CFTRI-MYSORE
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Introduction to

AN INTRODUCTION TO INDUSTRIAL MYCOLOGY

AN INTRODUCTION TO INDUSTRIAL MYCOLOGY

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BY

GEORGE SMITH
M.Sc., F.R.I.C.
RESEARCH DEMONSTRATOR, DEPARTMENT OF BIOCHEMISTRY,
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

FOREWORD BY

HAROLD RAISTRICK
Sc.D., F.R.S.
UNIVERSITY PROFESSOR OF BIOCHEMISTRY,
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

THIRD EDITION

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Introduction to

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PRODUCTION
WAR ECONOMY
STANDARD

THIS BOOK IS PRODUCED IN
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FOREWORD

It is a truism to say that were all types of micro-organisms suddenly to die out, human and animal life as we know it to-day would be impossible. Dead vegetable and animal remains are continuously being broken down by biochemical reactions brought about by micro-organisms, until their constituent elements are returned to the economy of nature as carbon dioxide, ammonia, nitrates, etc., to recommence the synthetic cycle for which the green plants are responsible. The part played by the lower fungi, or "moulds" as they are more commonly known, in this chain of degradative processes s one of paramount importance.

Further, in all industries handling organic materials, e.g. those concerned with food production, leather, textiles, wood, pharmaeuticals, etc., the danger of spoilage through growth of moulds is ne of which all those engaged in these industries are acutely aware. Thus the control and prevention of mould growth becomes a major problem to which, in the interests of increased efficiency, more and more attention is being given.

The harnessing of moulds for beneficial purposes, long established n some industries, as for example in the manufacture of Stilton, Gorgonzola and other types of cheese, has in recent years followed ome very interesting lines. Thus citric acid, until this century btained exclusively from the juice of citrus fruits, is now being made in thousands of tons per annum and in many different countries by growing chosen strains of the common black mould *Aspergillus niger* on sugar solutions, under carefully controlled conditions. The tudy of the biochemical changes, almost bewildering in their diverity, which can be brought about by moulds, is now a rapidly developing branch of biochemistry, and is attracting the attention f scientific workers in different parts of the world.

To those who are actively concerned in any of the industrial or cientific pursuits I have mentioned—and many more examples ould be given if these were needed—it is scarcely necessary for me o point out the paramount importance of some knowledge of the oulds themselves, and the more detailed and accurate this knowdge is, the better. Thousands of different species of moulds have een described and their differences in response to a particular nvironment, their tolerance of adverse conditions, and their biochemical characteristics, are almost as varied as their are great. There are in existence many admirable text- mycology which will probably meet the needs of the stu has had an adequate training in botany. But to those wit no botanical knowledge, and particularly for those who for the first time with an industrial problem of "mould' the lack of an adequate text-book setting forth in simple the facts of the subject, is a very real lack. To these an others who wish to acquire a first-hand knowledge of the "moulds" I must warmly recommend this book writte colleague, Mr. George Smith. The subject-matter of the b the basis of a course of lectures and practical work give author as part of the course to students working in this Scho post-graduate Academic Diploma in Bacteriology of the of London. Readers of Mr. Smith's book will, I think, fin to read and stimulating to study, and will, I hope and particularly appreciate, as I do, the really beautiful photomic which form a very important part of the book.

H. RAIS

London School of Hygiene
and Tropical Medicine.
July 1938.

PREFACE TO THE FIRST EDITION

THIS book is intended to assist those who are commencing the study of "moulds" rather than of fungi in general. There is already an extensive literature of systematic mycology, plant pathology and medical mycology, but there has been up to the present no book in English, apart from highly specialized monographs, dealing particularly with the fungi which are of importance in industry.

Sufficient general mycology is included to enable the student to follow up the subject in the standard text-books. The major portion of the book, however, consists of descriptions and illustrations of most of the genera of moulds which are of regular occurrence in ndustrial products, with more detailed consideration of the genera which are of greatest importance. Chapters on laboratory methods are sufficiently detailed to enable those who have had no previous biological training, and who are unable to get personal instruction, o work from the beginning along the right lines.

Many, probably the majority, of those who are called upon to undertake the solution of problems connected with moulds in ndustry are chemists, most of whom have had no training in botany and who find it difficult to learn the special terminology of mycological literature. Throughout the book, therefore, I have endeavoured o explain all such terms and usages as are like to be unfamiliar to he non-botanical reader.

All the figures, except Fig. 80, are from original photomicrographs, his type of illustration being, in my opinion, more suitable than ine drawings for the use of beginners. With few exceptions they are ll at certain precise and selected magnifications and are readily omparable one with another. I am grateful for permission to include ertain of my illustrations which have previously been published—igs. 25 and 26 in *The Journal of Botany*; Figs. 76, 79, 83, 84, 86, 1, 94, 95, 96, 99, 100, 101 and 106 in *The Journal of the Textile nstitute*; Figs. 6, 117 and 118 in *Transactions of the British Mycogical Society*.

To the many colleagues and workers in other institutions who have upplied infected materials and cultures, or who have made practical ggestions, I tender my thanks. I am also greatly indebted to essrs. Boardman & Baron, Ltd., of Great Harwood, for permission to make free use of the large number of photomicrographs taken i their laboratories.

G. S.

July 1938.

PREFACE TO THE THIRD EDITION

In the new edition of this book no major features have been change but a number of minor alterations and additions have been mad which it is hoped will make for greater clarity and usefulnes During the war years many interesting problems involving the act vities of moulds have arisen in various industries, but these canno be discussed freely until normal times return.

Eleven new illustrations have been added, of which four replac plates which were considered unsatisfactory. Owing to the pape restrictions it has been necessary, as in the second edition, to prin plates on both sides of the paper, and it has not always been possibl to arrange illustrations to face the relevant text.

Once again I tender my thanks to many colleagues and workers i other laboratories who have made suggestions for improvements.

May 1945. G. S.

CONTENTS

PAGE

FOREWORD v

PREFACE vii

LIST OF ILLUSTRATIONS xi

CHAPTER I. INTRODUCTION 1

Definition of mycology—Relation of fungi to other organisms—General morphology and classification—Oomycetes—Zygomycetes—Ascomycetes—Basidiomycetes—Fungi Imperfecti—Heterothallism

CHAPTER II. TERMINOLOGY AND CLASSIFICATION 15

Principles of classification—Meanings of terms—Rules of nomenclature—Pronunciation.

CHAPTER III. ZYGOMYCETES 19

The two orders, Entomophthorales and Mucorales—Key to genera of Mucorales—Descriptions of important genera and species

CHAPTER IV. THE ASCOMYCETES 33

General morphology—Classification—Important Genera

CHAPTER V. THE YEASTS AND RELATED FUNGI 41

General morphology—The True Yeasts—Classification—Descriptions of important genera and species—The non-sporing Yeasts—Methods of identification

CHAPTER VI. FUNGI IMPERFECTI 59

Definition of Fungi Imperfecti—Relationships to Ascomycetes—Classification—Saccardo's system—Melanconiales, Sphæropsidales, *Phoma*—Classification of Hyphomycetales—Vuillemin's system—Mason's scheme—Actinomycetes

CHAPTER VII. HYPHOMYCETALES 69

Key to industrially important genera—Descriptions of genera and important species

CHAPTER VIII. ASPERGILLUS 98

Industrial importance—Diagnosis—Generic limitations—Determination of species—Key—Descriptions of species

PAGE

CHAPTER IX. PENICILLIUM AND RELATED GENERA 129

Industrial importance—Difficulties of taxonomy—Diagnosis—*Gliocladium*—*Scopulariopsis*—*Pœcilomyces*—Classification of the *Penicillia* proper—Descriptions of the various sections and of important species

CHAPTER X. LABORATORY EQUIPMENT AND TECHNIQUE 164

Necessity for pure cultures—General equipment—Sterilization—Culture media—Methods of handling cultures—Methods of isolation and purification—Microscopic equipment and methods—Identification of species

CHAPTER XI. PHYSIOLOGY OF MOULD FUNGI 201

Food requirements—Respiration—Reaction of medium—Influence of light—Temperature relationships—Moisture requirements—Poisons—Influence of other fungi

CHAPTER XII. THE MAINTENANCE OF A CULTURE COLLECTION 212

Keeping moulds alive—Frequency of transfer and conditions of storage—Purity of cultures and sources of contamination—Mites—Maintenance of trueness to type

CHAPTER XIII. THE CONTROL OF MOULD GROWTH 225

Factory hygiene—Continuous sterility—Limitation of available moisture—Use of antiseptics—Testing of antiseptics—Details of various antiseptics

CHAPTER XIV. INDUSTRIAL USES OF FUNGI 239

Alcoholic fermentation and mould enzymes—Mould-ripened cheese—Oxalic and citric acids—Gluconic acid—Gallic acid—Glycerol—Fats—Proteins—Vitamins—Antibacterial substances—Miscellaneous products—Fungi and the soil

CHAPTER XV. MYCOLOGICAL LITERATURE 253

INDEX 261

LIST OF ILLUSTRATIONS

FIG. PAGE
1. Non-septate mycelium (*Rhizopus nigricans*), × 60 5
2. Germinating spores (*Aspergillus glaucus*), × 250 5
3. Septate mycelium (*Alternaria tenuis*), × 250 5
4. Oospheres (*Achlya sp.*), × 150 6
5. Oospores, × 150 6
6. Stages in zygospore formation (*Mucor sexualis*), × 100 9
7. Typical sporangium (*Rhizopus nigricans*), × 60 10
8. Clamp connections in mycelium of basidiomycete, × 500 10
9. Oval columella (*Mucor racemosus*), × 500 23
10. Pear-shaped columella (*Mucor sp.*), × 500 23
11. Chlamydospores (*Mucor racemosus*), × 100 23
12. *Mucor spinosus*—cymose branching, × 50 24
13. *M. spinosus*—sporangia, × 500 24
14. *M. spinosus*—columella, × 500 25
15. *Zygorhynchus Mœlleri*—zygospores, × 100 25
16. *Rhizopus nigricans*—rhizoids and stolons, × 50 26
17. *Rh. nigricans*—rhizoids and sporangium, × 50 26
18. *Absidia ramosa*—sporangium, showing apophysis, × 250 26
19. *Absidia spinosa*—young zygospores, × 250 26
20. *Thamnidium elegans*—young sporangiophore with sporangioles, × 50 29
21. *Th. elegans*—mature sporangiophore with sporangium, × 25 29
22. *Th. elegans*—sporangioles, × 200 29
23. *Syncephalastrum racemosum*—spore heads, × 250 30
24. *S. racemosum*—tubular sporangia, × 1000 30
25. *Byssochlamys fulva*—conidiophores, × 250 35
26. *B. fulva*—cluster of asci, × 500 35
27. *Monascus purpureus*—perithecium, × 250 36
28. *M. purpureus*—conidia, × 250 36
29. *Chætomium globosum*—perithecia, × 50 37
30. *Neurospora sitophila*—crushed perithecium, × 100 37
31. *Sordaria sp.*—perithecium in optical section, × 100 38
32. Typical Pyrenomycete—crushed perithecium, × 100 38
33. *Saccharomyces cerevisiæ*—vegetative cells, × 500 45
34. *Schizosaccharomyces Pombe*—vegetative cells, × 500 45
35. *Saccharomyces sp.*—ascospores, × 1000 46
36. *Zygosaccharomyces sp.*—relief stained, × 500 51

FIG. PAGE
37. *Klœckera apiculata*—relief stained, × 1000 51
38. *Phoma terrestris*—crushed pycnidium, × 250 61
39. *Mycelium sterilium* with sclerotia, × 20 62
40. *Actinomyces sp.*—conidiophores, × 200 62
41. *Oospora lactis*, × 250 73
42. *Oospora crustacea*, × 250 73
43. *Pullularia pullulans*, × 500 74
44. *Monilia sitophila*—young spore heads, × 200 75
45. *M. sitophila*—old spore heads, × 100 75
46. *Sporotrichum sp.*, × 250 76
47. *Cephalosporium sp.*, × 250 76
48. *Trichoderma viride*, × 500 77
49. *Verticillium cinnabarinum*—conidiophores, × 250 77
50. *Gliomastix convoluta*—conidiophores, × 500 81
51. *Botrytis cinerea*—conidiophores, × 100 81
52. *Trichothecium roseum*—conidiophore, × 200 83
53. *T. roseum*—spores, × 500 83
54. *Cladosporium herbarum*—portion of living colony, × 50 84
55. *C. herbarum*—young spore heads, × 250 84
56. *Stachybotrys atra*, × 500 85
57. *Helminthosporium monoceras*, × 90 85
58. *Alternaria brassicæ*—portion of living colony, × 42 86
59. *A. brassicæ*—chain of spores, × 250 86
60. *Alternaria tenuis*—spores from culture on poor medium, × 250 87
61. *A. tenuis*—spores from culture on rich medium, × 250 87
62. *Stemphylium sp.*—portion of living colony, × 65 88
63. *Stemphylium sp.*—spore cluster, × 500 88
64. *Stemphylium sp.*—septation of spores, × 500 88
65. *Isaria sp.*—colony on agar slope, × 1 91
66. *Isaria sp.*—branched coremium, × 12·5 91
67. *Stysanus stemonitis*—coremium, × 50 92
68. *Stysanus stemonitis*—young coremia, mounted, × 50 92
69. *Fusarium sp.*—portion of living colony, × 100 95
70. *Fusarium sp.*—spores, × 500 95
71. *Fusarium sp.*—spores, × 1000 95
72. *Fusarium sp.*—chlamydospores in mycelium, × 250 96
73. *Fusarium sp.*—chlamydospores in conidium, × 1000 96
74. Foot-cells of *Aspergillus*, × 500 101
75. Production of sterigmata in *Aspergillus*, × 500 101
76. Heads of *Aspergillus glaucus* with small vesicles, × 250 102

FIG. PAGE
77. Heads of *A. glaucus* with globose vesicles, × 500 102
78. *A. glaucus*—proliferation of sterigmata, × 250 105
79. *A. ruber*—perithecia, × 100 105
80. Ascospores of strains of *A. glaucus* (after Mangin) 106
81. *Aspergillus Amstelodami*—ascospores, × 1000 106
82. *A. restrictus*—portion of living colony, × 50 106
83. *A. restrictus*, × 500 109
84. *A. restrictus*—spores, × 1000 109
85. *A. fumigatus*—portion of living colony, × 50 110
86. *A. fumigatus*, × 250 110
87. *A. clavatus*, × 100 113
88. *A. giganteus*—single head in living culture, × 25 114
89. *A. giganteus*—typical clavate vesicle, × 100 114
90. *A. giganteus*—elongated vesicle, × 100 114
91. *A. nidulans*, × 500 115
92. *A. nidulans*—perithecia and Hülle cells, × 100 115
93. *A. versicolor*, × 500 116
94. *A. Sydowi*, × 500 116
95. *A. terreus*—portion of living colony, × 50 117
96. *A. terreus*, × 500 117
97. *A. flavipes*—columnar heads in old culture, × 50 118
98. *A. candidus*—freak head, × 300 118
99. *A. niger*—portion of living colony, × 20 121
100. *A. niger*, × 250 121
101. *A. Wentii*—mature heads in old culture, × 20 122
102. *A. Wentii*—young heads, × 25 122
103. *A. Wentii*, × 250 122
104. *A. ochraceus*—portion of living colony, × 20 125
105. *A. ochraceus*—single large head in culture, × 25 125
106. *A. ochraceus*, × 500 125
107. *A. effusus*, × 500 126
108. *Penicillium*—stages in conidium formation, × 1000 131
109. *Gliocladium roseum*, × 250 131
110. *Scopulariopsis brevicaulis*—spores, × 1000 132
111. *S. brevicaulis*—penicillus, × 1000 132
112. *Pœcilomyces varioti*, × 250 135
113. *Pœcilomyces*—typical sterigmata, × 800 135
114. *Pœcilomyces*—macrospores, × 500 135
115. *Penicillium spinulosum* (Monoverticillata), × 500 136
116. *P. luteum* (Biverticillata-Symmetrica), × 500 136

FIG. PAGE
117. *P. Charlesii* (Monoverticillata-Ramigena), × 500 139
118. *P. Charlesii,* × 500 139
119. *P. frequentans*—portion of living colony, × 50 140
120. *P. digitatum,* × 250 140
121. *P. digitatum,* × 500 140
122. Divaricate penicillus (*P. citrinum*), × 500 145
123. Divaricate penicillus (*P. Raistrickii*), × 500 145
124. *P. Raistrickii*—portion of living colony, × 100 146
125. *P. chrysogenum,* × 500 147
126. *P. roqueforti,* × 500 147
127. *P. brevi-compactum*—normal penicillus, × 600 148
128. *P. brevi-compactum*—very large penicillus, × 600 148
129. *P. janthinellum*—divaricate penicillus, from living culture, × 100 151
130. *P. nigricans,* × 500 151
131. Fasciculation at edge of young colony (*P. brunneo-violaceum*), × 25 155
132. *P. viridicatum,* × 500 155
133. *P. expansum*—single coremium, × 25 156
134. *P. expansum*—zonate and coremiform colony, × 0·75 156
135. *P. expansum,* × 500 156
136. *P. italicum*—colony with prostrate coremia, × 1 159
137. *P. italicum,* × 500 159
138. *P. funiculosum,* × 500 160
139. Phototropism in *Phycomyces nitens,* × 0·5 205
140. Set of plates—influence of one species on sporulation of another, × 0·35 206
141. *Penicillium* disease of *Aspergillus niger,* × 25 206
142. Mite in culture tube, × 100 217
143. Mite's eggs in culture tube, × 100 217

AN INTRODUCTION TO INDUSTRIAL MYCOLOGY

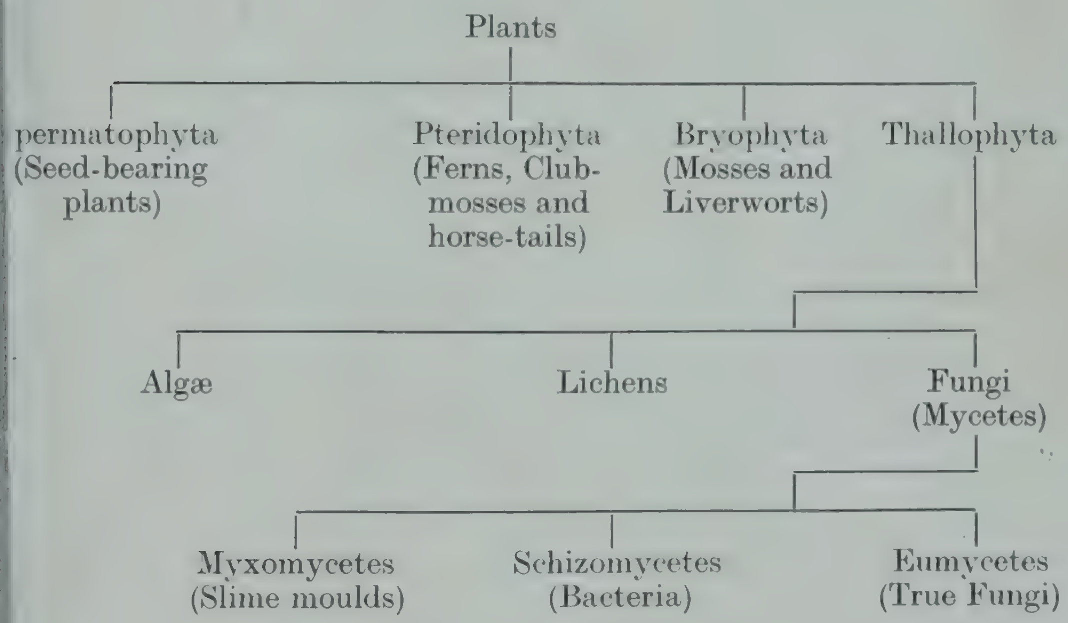
CHAPTER I

INTRODUCTION

Mycology is the science of the Fungi, an enormous group of organisms of very varied form and habit. The Fungi vary in size from the large toadstools, puffballs and bracket fungi to forms which are contained within a single cell of a plant; there are species which are edible and others which are amongst the deadliest of poisons; there are very rare species and forms which are to be found almost everywhere; some species are parasites of plants and animals whilst others inhabit the soil and break down dead organic material to form plant food; there are fungi which do incalculable damage to the products of man's labour and nearly related forms whose activities are harnessed for the production of substances of great commercial value. The fungi which are the concern of Industrial Mycology are a somewhat heterogeneous collection from amongst the various orders and families, most of them of common and widespread occurrence and nearly all of them of microscopic size.

The Fungi are usually classified as part of the plant kingdom and Table I shows their relationships to other forms of plant life.

Table I


Plants
Spermatophyta (Seed-bearing plants)
Pteridophyta (Ferns, Club-mosses and horse-tails)
Bryophyta (Mosses and Liverworts)
Thallophyta
Algæ
Lichens
Fungi (Mycetes)
Myxomycetes (Slime moulds)
Schizomycetes (Bacteria)
Eumycetes (True Fungi)

The Thallophyta are plants which show no differentiation int root, stem and leaf, but whose vegetative structure is known as thallus. This may be quite unsegmented, as in some of the simples fungi, or may show considerable specialization of structure wit corresponding specialization of function.

The Fungi are distinguished from the Algæ in showing a complet lack of chlorophyll, the green colouring matter which enables plant to synthesize carbohydrates from carbon dioxide and water in th presence of sunlight. Some mycologists, on this ground, regard th Fungi as organisms quite distinct from plants and belonging neithe to the plant nor animal kingdoms. Typical plants utilize simpl substances and build them up into substances of greater complexity in particular they utilize carbon dioxide, to form starch, cellulose fats and the like, and liberate oxygen. Animals, on the whole, brea down organic matter from the complex to the simple and diffe fundamentally from plants in that they absorb oxygen and liberat carbon dioxide. Fungi resemble plants in structure and in lack o means of locomotion, but are more related to animals in that the require oxygen and that, invariably, one of the products of metabolism is carbon dioxide. A good introduction to this question o phylogeny is given in a popular publication by Ramsbottom (1929) and the subject is further developed in most of the monographs o systematic mycology. It therefore need not be elaborated here.

The Lichens are compound organisms, consisting of algæ an fungi in symbiotic relationship. Their study is a separate branc of science, since the fungus-alga association is so close that the Lichens may be classified into genera and species just as if they were single organisms, and the fungi themselves are unknown apart from their algal associates. Those who are interested in this group should consult the excellent monograph by Miss A. L. Smith (1918, 1926) or her text-book (1921).

The Bacteria, comprising, in the above table, one of the thre classes of fungi, are the province of the bacteriologist. Whether the belong with the fungi at all is a moot point, but their consideratio is outside the scope of this book.

The Myxomycetes have a somewhat varied history, having bee claimed by both botanists and zoologists. The consensus of presen opinion is that they are certainly not fungi and are probably classe more correctly as Mycetozoa. There are several monographs dealin with these interesting organisms, the best for British students bein that of Lister (1925).

Mycology then, in its modern sense, is concerned only with the Eumycetes or true fungi.

General Morphology and Classification

Most of the common mould fungi may be propagated by transferring any part of the plant to a fresh substratum, but the normal development of a thallus commences with the germination of a *spore.* Germination is the term commonly employed, but its use n this connection must not be confused with the more accurate use of the word as applied to seeds of ordinary plants. Spores of fungi are of different shapes and sizes, they may be one- or many-celled, but in no case does a spore contain a true germ or plant embryo. A spore consists of an outer wall, the epispore, which may be smooth, pitted or roughened by small projections, and an inner wall, the endospore, enclosing a mass of protoplasm in which drops of oily or fatty material and, with special staining methods, one or more nuclei may be distinguished, but which is otherwise little differen-iated. On reaching a favourable situation the spore swells and then puts out one or more ***germ-tubes.*** The germ-tube elongates and, n most cases, becomes richly branched, forming a network of filaments (***hyphæ,*** singular ***hypha***) collectively known as a ***mycelium,*** vhich may penetrate the substratum, grow on the surface or may e partly submerged and partly aerial. Later, in all but a few cases, he fungus provides for propagation of the species by putting forth pore-bearing hyphæ or by building up special fruit-bodies in which he spores are formed. The methods of spore formation and the haracteristics of the spores and of the spore-bearing organs form e basis of all systems of classification of fungi.

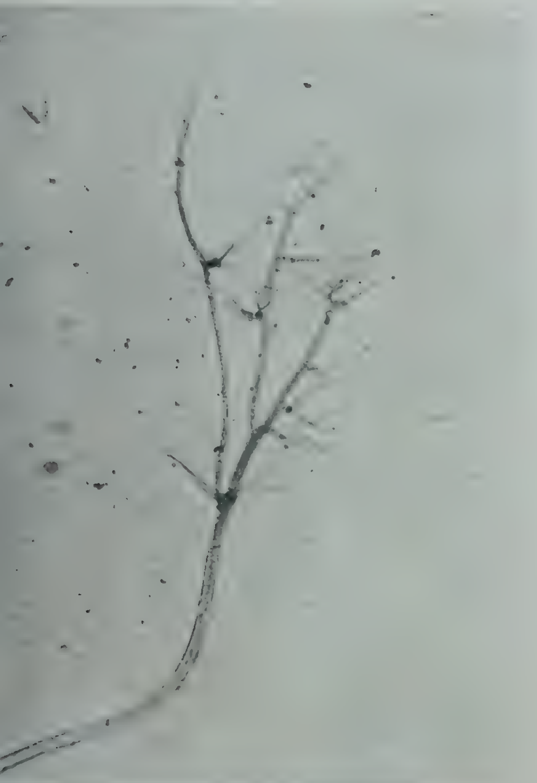
There is no uniformity as regards classification. The terms used denote rank, such as class, order, alliance, family, are used in rious senses by different authorities and the boundaries of the oups are by no means equivalent in the usages of various authors. obably the most useful system for the beginner and for the tech-logist is that adopted in Rabenhorst's Kryptogamenflora and is system will, in the main essentials, be followed throughout the esent book.

The Eumycetes are divided into 2 main groups and these int 5 classes, as follows:

I. Hyphæ typically non-septate . .	PHYCOMYCETES.
A. Sexual fructification by oospores; asexual spores often motile . .	Class 1, OOMYCETES.
B. Sexual fructification by zygospores; asexual spores non-motile, borne in sporangia or as conidia . .	Class 2, ZYGOMYCETES.
II. Hyphæ typically septate . . .	MYCOMYCETES.
A. Principal spores endogenous, in asci; accessory fruit forms often present	Class 3, ASCOMYCETES.
B. Principal spores exogenous, on basidia	Class 4, BASIDIOMYCETES.
C. Asci and basidia unknown; propagation by asexual spores only .	Class 5, FUNGI IMPERFECTI.

Several of the terms used require explanation. The first, mai sub-division of the Fungi is based on the presence or absence o cross-walls (*septa*) in the hyphæ. In the Phycomycetes septa ar rarely present except in the fruiting organs, though they are occasionally found in old cultures. Fig. 1 shows vegetative hyphæ of species of *Rhizopus*, almost root-like in appearance, the whole bein part of one enormously elongated and branched cell. If a young vigorously growing culture of the very common mould, *Rhizopu nigricans*, preferably growing in a Petri dish, be examined with low power of the microscope, it will usually be noticed that th cell contents are streaming along both the main hyphæ and th secondary branches, indicating clearly the entire absence of septa In the Mycomycetes the vegetative hyphæ usually begin to develo septa at a very early stage. Fig. 2 shows germinated spores o *Aspergillus glaucus* and the septa in the young germ-tubes a clearly visible. Fig. 3 shows the richly septate mycelium of anothe common mould, *Alternaria tenuis*.

The next basis of subdivision is the method of formation of t spores and here it is necessary to understand that the term "spore is used in mycological literature with various meanings. In i wider sense it includes all the types of specialized cells, formed or within, or cut off from, special organs, and obviously designed a means of propagation and dissemination of the species. In narrower sense, that used by the systematist, it is restricted to me the product of a sexual process. The sexual union may be obviou as in the Zygomycetes, but it is more often obscured and has on



IG. 1.—Non-septate mycelium. *Rhizopus nigricans.* × 60.

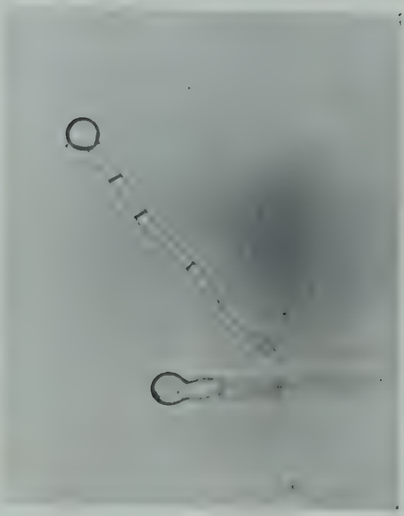


FIG. 2.—Germinating spores of *Aspergillus glaucus,* showing septation of primary hyphæ. × 250.

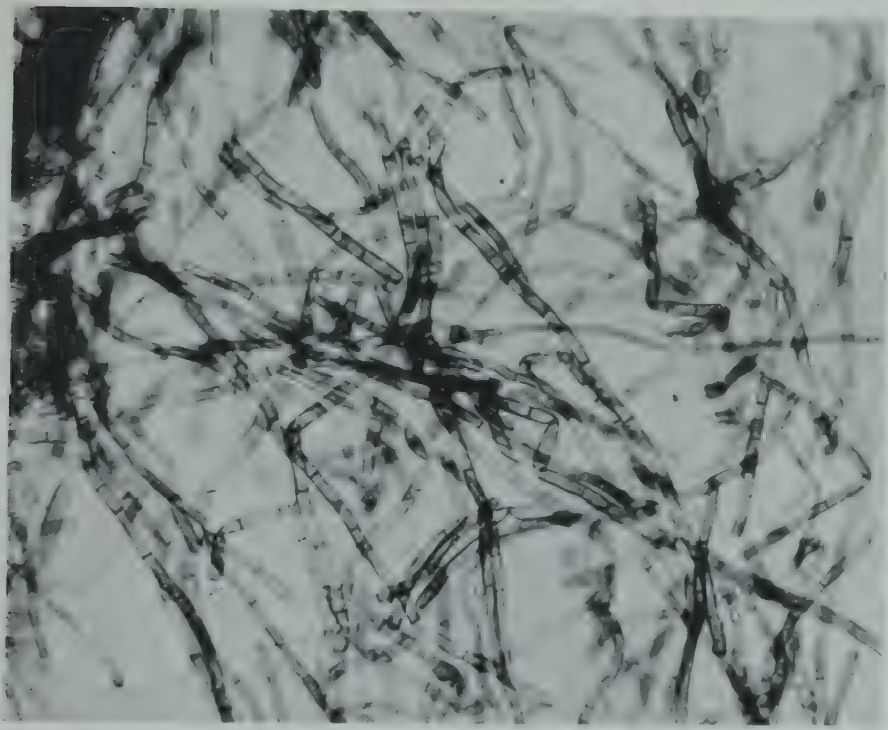


FIG. 3.—Septate mycelium—*Alternaria tenuis.* × 250.

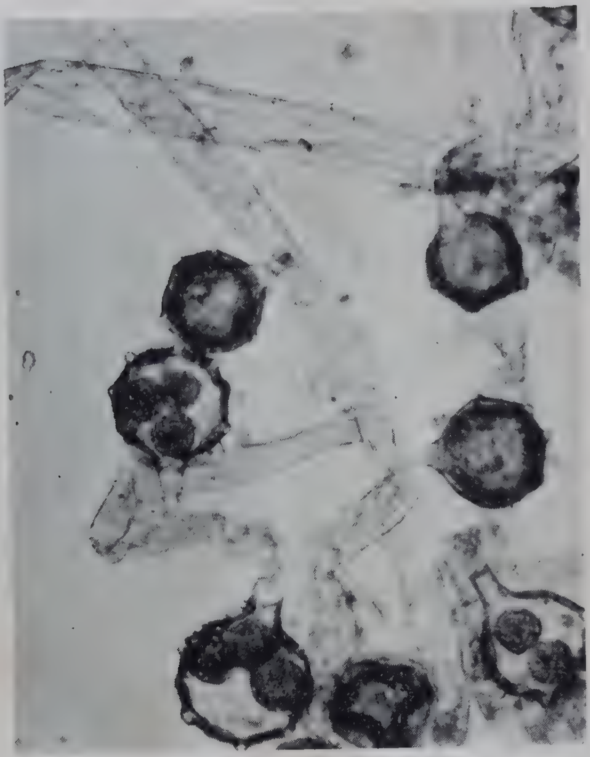


Fig. 4.—*Achlya sp.*—Oogonia with oospheres. × 150.

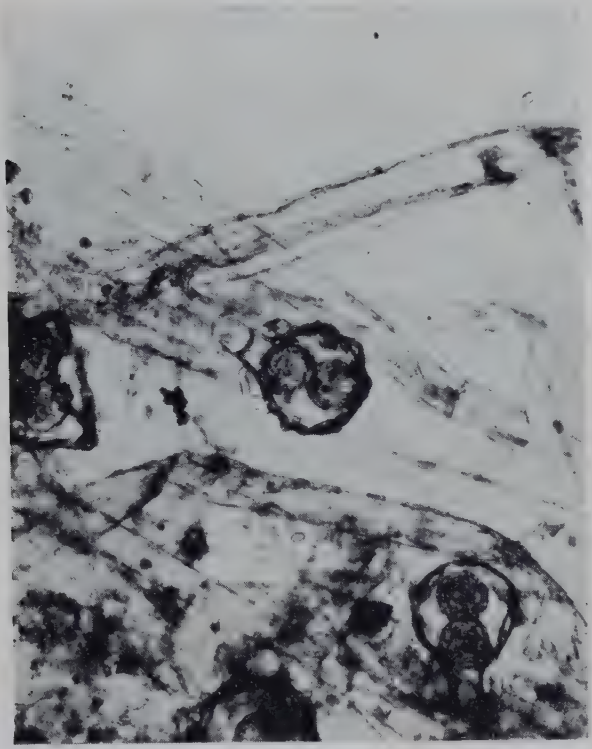


Fig. 5.—*Achlya sp.*—Oogonia with oospores. × 150.

been elucidated by cytological studies, although the presence of fruit-bodies bearing a definite number of spores, usually a power of 2, is usually an indication that a sexual process is involved.

Asexual or accessory spores are of various forms, are borne on very varied types of spore-bearing structures and, unlike the sexual spores, are produced in indefinite numbers. The mode of occurrence and the size, shape, septation and markings of the asexual spores are of generic significance, even in the orders which are classified primarily by sexual spores and, in the great group of Fungi Imperfecti, which includes most of the common moulds, are the basis of all systems of classification. The following list includes most of the special terms which are in use to designate different types of asexual spores and the organs which bear them.

Chlamydospores. Thick-walled resting spores, formed by swelling and thickening of single short cells in submerged vegetative hyphæ, in aerial hyphæ, or even in multiseptate conidia such as those of species of *Fusarium* (see Figs. 11, 72, 73).

Conidia (sing. *Conidium*). Spores formed by budding or abscission from special hyphæ (conidiophores). Some authorities restrict the term to the spores cut off successively from sterigmata (*q.v.*), but others include spores formed in a variety of ways.

Conidiophore. A specialized hyphæ bearing conidia either at the tip or, more rarely, along its length. In some genera the conidiophore terminates in a series of branches, of more or less definite form, on which the conidia are borne.

Coremium. An erect compact cluster of conidiophores. The conidiophores may be of different lengths, the coremium being then more or less cylindrical and clothed with spores throughout the greater part of its length, or may be of approximately equal length, in which case the coremium has a distinct stalk and a spore-bearing head (see Figs. 65–68 and 133).

Oidia. A term usually used only in the plural (*Oidium* is the name of a genus of fungi). In some fungi portions of the mycelium form close series of very short cells, which may remain roughly rectangular or may round up to resemble a chain of beads. The mycelium then breaks up at the septa and the fragments, known as oidia, function as spores (Figs. 41, 42).

Pycnidia (sing. *Pycnidium*). Fruit-bodies which are typically flask-shaped, but are often irregular, mostly of a size readily visible to the naked eye, lined with short conidiophores. When crushed the spores emerge as an irregular mass (Fig. 38).

Sclerotia. Neither spores nor spore-bearing structures, but compacted masses of mycelium, often very hard, varying in size (in mould fungi) from a fraction of a millimetre to several millimetres in diameter. Some of the higher fungi produce very much larger sclerotia (Fig. 39).

Sporangium (pl. *Sporangia*). A closed receptacle, usually round or pear-shaped, borne on a stalk (the *sporangiophore*) and containing an indefinite number of spores. The only mould fungi which produce spores in sporangia are the Mucorales (Chapter III).

Sporodochium. A cushion-shaped, tightly packed cluster of short conidiophores.

Sporophore. Any spore-bearing structure. A conidiophore is a special type of sporophore.

Sterigma (pl. *Sterigmata*, also called *Phialides*). A specialized terminal cell, roughly cylindrical in the basal portion, usually with a constricted tip which continually elongates, but from which conidia are successively cut off so that the length of the cell remains sensibly constant.

Stroma (pl. *Stromata*). As formed in artificial cultures, is a gelatinous or leathery layer covering the surface of the culture medium and bearing spores on very short conidiophores.

The Oomycetes. In this class the sexual organs are readily distinguished as ***antheridium*** (the male organ) and ***oogonium*** (female organ). The oogonium, when ripe, shows part of the protoplasm aggregated into one or more ***oospheres*** (see Fig. 4). It is fertilized by the antheridium coming in contact with it or, in one family, the Monoblepharidaceæ, by motile male cells liberated by the antheridium. After fertilization the oospheres round up to from the ***oospores***, which lie free within the oogonium (Fig. 5). Most Oomycetes also produce sporangia containing asexual spores. Many species are aquatic or grow in very damp situations and, the better to aid rapid dissemination, produce motile spores known as zoospores. The order includes a number of important plant parasites and a few parasites of animal life, the best known being species of *Saprolegnia* on fish and on insects in water, but none of the species is likely to be encountered in industrial work and, therefore, their consideration will be limited to this brief statement of their systematic position.

The Zygomycetes. In this class the sexual organs are not distinguishable as antheridium and oogonium. They may be similar in size (the usual case), or regularly dissimilar as in *Zygorhynchus*, but they are essentially alike in nature and function. Whereas in

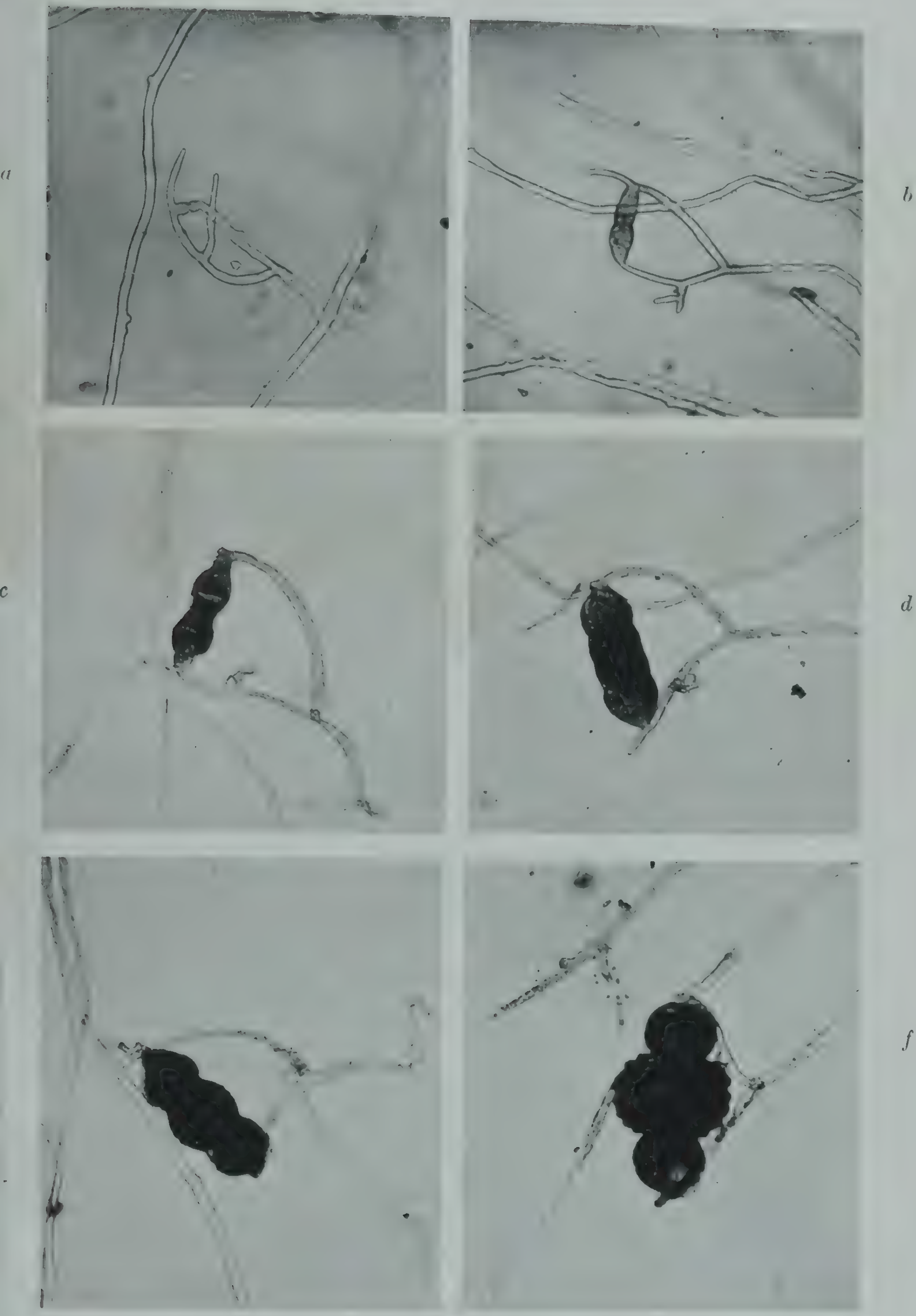


FIG. 6, *a–f*.—Six stages in zygospore formation—*Mucor sexualis*. × 100.

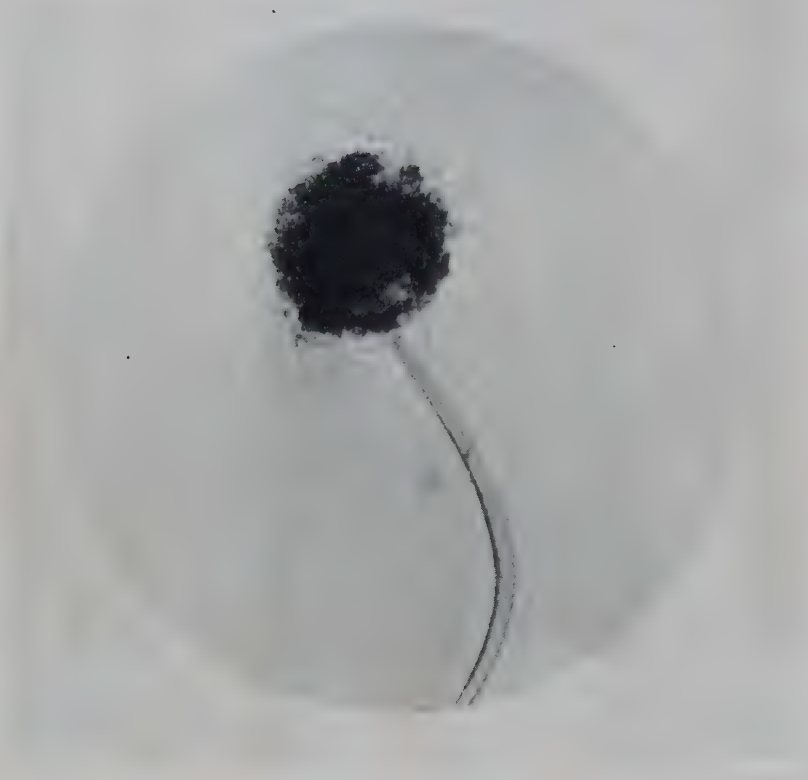


Fig. 7.—Sporangium of *Rhizopus nigricans*. × 60. The sporangial wall has burst to liberate the spores.

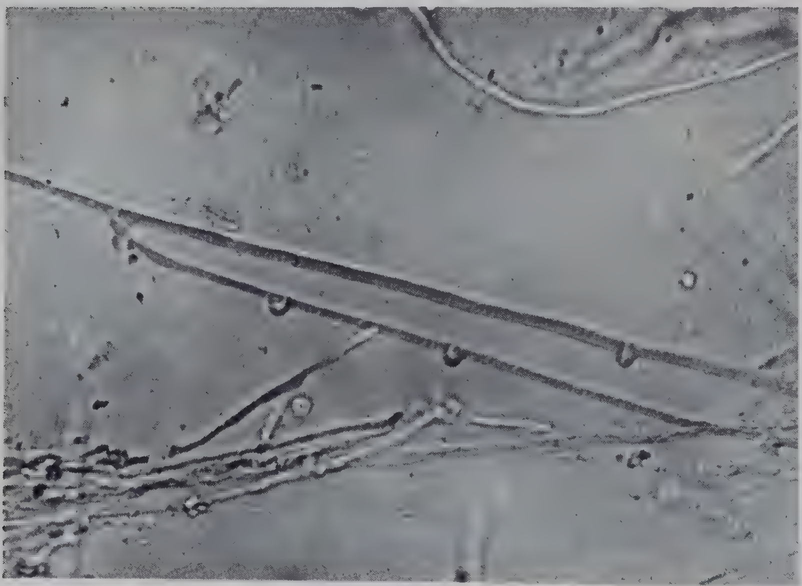


Fig. 8.—Clamp connections in mycelium of a Basidiomycete —*Polyporus sp.* × 500.

the Oomycetes the oospore is formed within the oogonium and is thus termed endogenous, the *zygospore* results from the fusion of two cells cut off from the sexual organs and is thus exogenous. Two hyphæ touch and at the point of contact develop protuberances, the *progametangia*, which gradually elongate and swell, remaining in contact themselves whilst pushing the parent hyphæ further and further apart. Septa next appear in each progametangium, the cut off portions which remain in contact being the *gametangia*. The latter fuse, the cell walls in contact disappearing, and the fusion cell becomes rounded and, usually, thick walled and dark coloured. Fig. 6 shows six stages in zygospore formation. A slight variant of this process is found in *Piptocephalis*, where the zygospore is formed in a bud put out from the cell which is produced by fusion of the gametangia. In some genera the zygospore is surrounded by stiff hairs or by a web of protecting hyphæ, whilst in others it lies free amongst the aerial hyphæ. In several genera which obviously belong here, zygospores have never been observed.

Asexual spores are non-motile and, in the best-known genera, are produced in globose sporangia borne on special hyphæ, the sporangiophores. Fig. 7 is a photograph of a typical sporangium of *Rhizopus nigricans*. In several genera sporangia are lacking and asexual propagation is by conidia.

The Zygomycetes include about 200 species, amongst which are a number of moulds of very common occurrence and considerable economic importance. They are considered in more detail in Chapter III.

The Ascomycetes. The typical fruit-body is a closed sac, the *ascus*, containing usually 8 spores, more rarely a smaller or larger number. The group shows a gradual transition from primitive forms, which produce single, naked, globose asci to species which build up elaborate fruit-bodies with large numbers of club-shaped asci arranged in parallel series. The details of ascus formation are not readily observed and in some cases are not completely understood at the present day. Full treatment of the group from this standpoint is to be found in several modern works on systematic mycology. Asexual reproduction is by conidia, which show great diversity of form and arrangement, or by oidia. The class comprises about 15,000 species and includes both saprophytes and parasites, microscopic species and large fleshy fungi, some of which are edible. The genera which are of importance to the industrial mycologist are considered further in Chapter IV.

The Basidiomycetes. The spores which give the name to the group, the *basidiospores*, are borne exogenously on special organs, the *basidia.* In the typical species each basidium bears four spores. In the higher Basidiomycetes, the mushrooms and toadstools, the basidia are found in serrated ranks on the gills of the large fleshy sporophores and there are often specialized arrangements for ensuring the widespread distribution of the spores. The lower Basidiomycetes, including the destructive parasites known as smuts and rusts, have a more complicated life cycle, and their position in a scheme of classification is not quite so obvious. Since most of these are obligate parasites, no attempt will be made here to discuss their relationships and life histories. They have been very extensively studied and are treated in detail in numerous monographs (see Chapter XV). A few of the more primitive species are occasionally encountered in artificial culture, but the growths are atypical. Many may be recognized as belonging to this Class of Fungi by the presence, in the mycelium, of what are known as clamp connections. These peculiar structures are found only in the Basidiomycetes. A little distance from the tip of a growing hypha a very short branch appears; this curls backwards and reunites with the parent hypha close to the point of emergence; in the meantime a septum is formed midway in the hypha and another one at the base of the hook. These structures are readily detected, as slight bulges on the hyphæ, under a low power of the microscope, but require a high power for resolution of details (Fig. 8).

In addition it has been found that certain unicellular, yeast-like fungi, at first regarded as related to the true yeasts, and hence to the Ascomycetes, are in reality primitive Basidiomycetes (see Sporobolomyces, p. 52).

The Fungi Imperfecti. This group comprises a very large number of fungi which have incomplete or incompletely known life cycles. Most of them are probably Ascomycetes which produce the perfect stage only under special conditions which have not yet been discovered, or which have entirely lost the power of producing asci. It is now known with certainty in a few cases that heterothallism exists amongst the Ascomycetes (see below) and it is probable that this phenomenon accounts for many other cases of apparent loss of sexuality. In the absence of perfect fructifications, on which alone a rational classification can be based, it is necessary to classify the Fungi Imperfecti on characters of the asexual spores. According to the systematists, the genera thus set up are not true genera at all, since it is known, on the one hand, that a single genus of Ascomycetes may include species with very different types of imperfect fructifications and, on the other, that the conidial forms of species belonging to widely different genera may be very similar. It follows, therefore, that a so-called "form genus," comprising a number of species with the same type of conidial fructification, may be a purely artificial grouping of fungi which are really quite unrelated. However, until real relationships are discovered, it is necessary to have a convenient classification of the enormous number of Fungi Imperfecti, as otherwise identification of any given form would be a hopeless task.

The great majority of the common fungi which are popularly known as moulds or mildew belong in this group and will be dealt with at greater length in Chapters VI to IX.

Heterothallism. It was for long a puzzle to mycologists why some species of the Mucorales regularly produced zygospores whilst others seemed to lose the power on continued culture and in still other species, which were obviously nearly related, zygospores were quite unknown. Various theories attempting to explain the matter on a nutritional basis failed to take account of all the facts and it was not until 1904 that the riddle was solved by Blakeslee (1904, 1920). He showed that the sporangiospores from a strain of *Mucor* which was producing abundant zygospores gave rise, on germination, to two distinct strains of mycelium, called by Blakeslee + and —. Each strain, by itself, was incapable of giving zygospores, but if both strains were grown in such a way that the two mycelia could touch, zygospores were produced along the line of contact. It was thus obvious that mass transfers from a culture containing zygospores would usually result in a mixed culture of the + and — strains and zygospores would again be produced, but that single spore cultures would invariably lack zygospores and that sub-cultures made by transfer of a few spores would very often result in a pure culture of one strain only. Such species, where sexuality resides in a whole thallus, are called ***heterothallic***. In contrast to these the ***homothallic*** moulds produce zygospores by contact of branches from the same mycelium and often from the same hypha. The illustrations of zygospore formation, Fig. 6, obviously depict a homothallic species. Both + and — strains of a number of heterothallic moulds are known and can be obtained from the various collections of type cultures, but, in a great many cases, only one strain has been found and hence zygospores have never been observed.

Heterothallism is most frequently encountered in species belonging in the Mucorales and it is to the numerous studies of such species that our knowledge of the phenomenon is chiefly due, but in recent years it has been shown that it is not confined to this order. Several Ascomycetes have been found to be heterothallic and in certain of the Basidiomycetes a still more complicated state of affairs exists, the four spores from a single basidium being all of different "sexes."

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CHAPTER II

TERMINOLOGY AND CLASSIFICATION

Readers who have already had some training in one of the biological sciences will have no difficulty with the system of nomenclature used by mycologists, but, to anyone without previous experience of this kind, names are apt to appear irrational and the different usages of various authorities confusing. This chapter is intended to help the beginner to understand the method which underlies the strangeness and apparent uncouthness of many mycological terms, and, at the same time, to give a broad outline of the special terminology and basis of classification.

The "unit" of any scheme of classification is the species. What exactly constitutes a species is one of the vexed questions of biology and need not be gone into, but for practical purposes, it may be taken that the term is used to denote any organism which shows recognizable and constant differences in structure from all other organisms, except, of course, from other individuals which are of the same species. A genus groups together a number of species which show a closer resemblance to each other than they do to any other species. On the same principle genera are classified into families, these into orders and orders into classes. Such a scheme of classification serves a twofold purpose. From the purely scientific point of view it represents an attempt to show how all the various species are related to each other and to other living things and it also provides a means of identification of species. Up to a point mycologists are agreed as to how the Fungi should be classified, but there are many minor points of variance between the different authorities and more fundamental differences as regards a few groups. This state of affairs is due, partly to gaps and imperfections in our knowledge, and partly to personal differences in interpretation of observations made under difficult conditions. Also, it must be realized that there are few sharp dividing lines in Nature, almost all the groups of fungi merging imperceptibly into adjacent groups, with the result that it is often a matter of opinion as to where exactly a dividing line should be placed.

Nomenclature

Every species bears two names, that of the genus to which it belongs and its own specific name. The generic name is a Latin substantive and is always written with a capital letter, whilst the species name is adjectival, agrees with the substantive in gender and case and, normally, is not capitalized. Names should be, and frequently are, to some extent descriptive. Thus the name *Penicillium* means "broom-like" and describes the appearance of the fruiting heads of typical species of the genus; *Aspergillus* was named from a resemblance of the spore heads to the Aspergillum, a kind of mop used for distributing holy water (Latin, *aspergere* = to sprinkle); *Helminthosporium* is a genus of fungi with elongate, multi-septate spores which have a somewhat fanciful resemblance to worms (Helminthes); *Rhizopus* is characterized by its production of root-like structures known as rhizoids. Specific names usually refer to minor characteristics such as colour, odour or growth habit. To illustrate from a single genus, *Penicillium purpurogenum* produces a pigment which colours the culture medium a bright purple, *P. roqueforti* is the mould used for ripening Roquefort cheese, *P. suaveolens* gives rise to a pleasant odour, whilst *P. lanosum* forms a floccose mass of mycelium which is distinctly woolly in appearance.

Many specific names, and not a few names of genera and of families, have been bestowed in honour of well-known mycologists. Other names of species are derived from place-names or, in the case of parasitic fungi, from generic names of host plants. There are two ways of using proper names as names of species. The more usual way is to use the genitive case of a Latinized form of the name, as *Aspergillus Wentii* and *Penicillium Daleæ*, from Went and (Miss) Dale respectively. A less common method is to use the inflected ending -anus, -ana, -anum, as *Puccinia de Baryana* and *Heterosporium Magnusianum*. Proper names used thus are mostly written with capital letters, although there is no uniformity as regards this practice, some authorities capitalizing personal genitives but not names derived from host genera, whilst others do not use capitals for any specific name. Proper names convey no meaning and are thus less satisfactory than descriptive names, but it is certainly very tempting to use them for new species in a genus which is already a very large one and in which nearly all the possible descriptive names have already been used, and they are undoubtedly more distinctive than a long series of binomials of colour. The custom of using generic names of host plants has unfortunately led to the reprehensible practice, not uncommon, of creating a new species every time a fresh host is found for a particular genus. Scores of species have thus been founded without any justification from adequate experimentation, with the result that some genera are hopelessly cumbered with a mass of names which ought to be relegated to the synonymy.

Family names are commonly derived from the names of the most important or most typical genera included therein. All names of families end in -aceæ (pronounced -ay′se-ee). Ordinal names, in modern usage, end in -ales (pronounced -ay′lees), but in much of the older literature the ending -ineæ is used. All names of higher rank end in -etes, pronounced as one syllable. Thus the Phycomycetes include the class Zygomycetes, one order of the latter is the Mucorales, the largest family of which is the Mucoraceæ including the genus *Mucor*. Names of classes, orders and families are capitalized but are not printed in italics. Sub-orders, sub-families, etc., are sometimes created to assist identification and rational grouping and bear names with ordinal and family endings respectively.

It is usual, when citing the name of a species, to append the name of the author who published the original diagnosis (i.e. description). The name of the authority is not written in italics. In some cases a new species is described by one worker as belonging in a certain genus and is later transferred by another author to a different genus. Both names are then given, the name of the original describer first, in brackets. For example, *Eurotium Chevalieri* Mangin is now regarded as a species of *Aspergillus* and becomes *Aspergillus Chevalieri* (Mangin) Thom and Church. Authors' names are frequently abbreviated, or even given as initials only, as Sacc. for Saccardo, Cda. for Corda, Wr. for Wollenweber, DC. for de Candolle, B. and C. for Berkeley and Curtis, C. and E. for Cooke and Ellis.

Pronunciation

The pronunciation of names of fungi is, in this country, in accordance with the traditional English method of pronouncing Latin, with the vowels mostly as in common English words. A terminal -i is long as in "like"; a terminal -ii has the first -i short, as in "tin" and the second long; "ae" is pronounced "ee"; "g" and "c" are soft before "e," "i" and "y" and hard before other vowels, and so on. Endings such as -genum and -ferum, derived from Latin verbs, retain their original accent on the first of the two syllables. To give a few typical examples: chrysogenum pronounced krī′so-jēe′num, the "y" and "e" being both long an accented; *Sphærotheca* is sfēe′ro-thēe′ka whilst *Cephalothecium* se′fal-o-thee′sium; fungus has a hard "g" whilst the "g" in fun is pronounced as "j"; the "c" is hard in ascus but soft in th plural, asci. The rules given are those most commonly observed, bu some mycologists modernize many names, particularly as regar accenting.

Some knowledge of Latin is essential to any student of mycolog for, not only is it useful to be able to appreciate the meanings names, but many important publications are entirely in Latin an it is now a rule, made by an International Congress of Botanists 1930, that every description of a new species, in whatever languag it is published, must be accompanied by an adequate diagnosis Latin.

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CHAPTER III

ZYGOMYCETES

THE essential features of the Zygomycetes have already been outlined in Chapter I and need not be further elaborated here. Most of the commoner forms are readily recognized as belonging to the group by their characteristic mode of growth, colonies being usually loosely floccose and of a grey or brownish grey colour. Two genera, *Mucor* and *Rhizopus*, include the great majority of the species which are usually encountered in the laboratory, but members of several other genera are found sufficiently frequently to justify their inclusion here. Zygospores have been found in comparatively few species and hence are not made the main basis of classification. However, although so many species lack the perfect fruiting stage, there has never been any question of placing these with the Fungi imperfecti, owing to the very characteristic type of thallus which distinguishes the Class.

The Zygomycetes are divided into two orders as follows:

Asexual spores occurring in sporangia . .	MUCORALES.
Asexual spores as conidia, borne on specialized conidiophores and forcibly shot away at maturity	ENTOMOPHTHORALES.

The Entomophthorales are mentioned only for the sake of completeess since most of the species are obligate parasites on insects and nly a very few have ever been grown in artificial culture. The rgest and best-known genus is *Empusa*. *E. Muscæ* Cohn is of ommon occurrence in the late summer, when it forms characteristic hite haloes round the bodies of dead flies.

Mucorales

The great majority of the species are saprophytic, occurring on wide variety of organic substrata, whilst the remainder are parasitic n other members of the order. The typical thallus consists of oarse hyphæ growing loosely, white in the early stages of growth nd becoming grey or brownish with the production of the fruiting ructures. The usual mode of asexual reproduction is by spores roduced in large numbers in globose sporangia, borne on special hyphæ (*sporangiophores*) which may be simple or branched in various ways. In most genera the tip of the sporangiophore i swollen, the swollen end (the *columella*) projecting into the sporangium. The columella may be of various shapes, globose, ovoid hemispherical, etc. (Figs. 9, 10, 14), and has diagnostic importance in determination of species. The sporangial wall may be thin, when the spores are liberated by its rupture or dissolution, or may be cutinized and shot off, or broken off, in one piece. In a number of families various modifications occur of the typical globose, many-spored sporangium, the most important of these being described below.

A number of different classifications of the Mucorales have been proposed, but the total number of genera is not large and, in the great majority of cases, the identification of a fungus as far as its genus is a fairly simple matter whichever key is used for the purpose The following simple key ignores division of the order into families and includes all the genera which are commonly found on industria products. Many of the remainder live on dung, and although of widespread occurrence, are unable to grow on ordinary culture media.

KEY[1] TO THE COMMON GENERA OF MUCORALES

1.	Sporangia tubular, radiating from a vesicular swelling	*Syncephalastrum*.
	Sporangia globose or nearly so . . .	 2
2.	Many-spored sporangia and few-spored sporangioles present	*Thamnidium*.
	Sporangia alone produced . . .	 3
3.	Sporangiophores stiff, dark coloured, metallic in appearance	*Phycomyces*.
	Sporangiophores hyaline or nearly so .	 4
4.	Rhizoids and stolons present . . .	 5
	Rhizoids and stolons absent . . .	 6
5.	Sporangia globose, sporangiophores arising from points of attachment of the rhizoids	*Rhizopus*.
	Sporangia pear-shaped, sporangiophores mainly as branches from the stolons .	*Absidia*.
6.	Homothallic, zygospores with very unequal suspensors	*Zygorhynchus*.
	Homo- or heterothallic, zygospores with approximately equal suspensors . .	*Mucor*.

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[1] In the dichotomous system, used for this key and others later in the book (al used by many other authors), all the essential data required for identifications a arranged as *pairs* of contrasted characters, the pairs being numbered consecutive on the left. Each member of a pair leads, on the right, either to the name of a genu or to another higher number, i.e. to a further pair of contrasted features.

Mucor is the largest genus of the order and determination of species is far from easy. The criteria on which identifications are based are the mode of branching, if any, and the length and diameter of the sporangiophores, the colour and size of the sporangia, the character of the sporangial wall, the size and shape of the columellæ, the size and shape of the spores, the characteristics of zygospores and chlamydospores, if any, and general colony characteristics, such as colour and height of aerial growth. About 150 species have been described in Saccardo's Sylloge, but many of these are rare and some are of doubtful standing. Lendner (1908) describes 51 species, including the majority of those which are likely to be met with in most kinds of work. His monograph has been widely used by taxonomists and his key has been reproduced in several later publications, as for example the text-books of Harshberger and Henrici see Chapter XV). Since the appearance of Lendner's book Hagem (1910) has described 4 new species, Povah (1917) has added 6, and odd species have been described by a number of other workers. Until recently, however, identification of species, even with all the relevant literature available, has been difficult and often unsatisactory. The probable reason is that in *Mucor*, as in other common genera of moulds, certain ubiquitous species exhibit minor differences between different strains. Taxonomists who have handled comparatively small numbers of strains have not sufficiently appreciated this fact and hence have not always distinguished between true and spurious specific characteristics. Most original diagnoses have been drawn in terms insufficiently broad to cover these strain differences, with the result that workers handling *Mucors* have but rarely found trains which tallied exactly with published descriptions.

In a recent work on the Mucorales. Zycha (1935) has considerably implified the taxonomy of *Mucor* by taking into account this atural variation. The total number of species has been reduced 42 and, of these, several, of which type cultures are not available, re accepted only provisionally. Zycha's monograph is easily the most satisfactory treatment up to date both of this genus and of he remaining genera of Mucorales, and seems likely to come into eneral use by taxonomists.

A still more recent treatment of the order is by Naumov (1939). He includes a number of genera not recognized by Zycha and rejects thers which Zycha considers to belong to the Mucorales. The genus *Mucor* is divided into 13 sections, or sub-genera, and the total umber of admitted species is 88. However, the majority of the

46 names not included in Zycha's list are regarded by the latter author as synonyms. The whole of the diagnoses in Naumov's book are incorporated in the keys, and this makes the work less satisfactory to use than Zycha's, in which short keys and separate diagnoses of all species are given.

M. mucedo Linné is the type species of the genus, having first been described in 1762. It is found chiefly on dung, where it forms erect sporangiophores up to 15 cm. long, with greyish sporangia encrusted with crystals of calcium oxalate. In culture the sporangiophores are usually shorter and bear at the base short branches terminating in small sporangia. The terminal sporangia are 100–200μ in diameter, with diffluent wall; columellæ pear-shaped to cylindrical, often with orange-coloured contents; spores elliptical, 6–12μ long.

M. piriformis Fischer is of fairly common occurrence in any kind of damp situation. It forms dense, cottony, grey colonies, 2–3 cm. high, with sporangiophores mostly unbranched, up to 50μ in diameter. Sporangia are globose and very large, 250–350μ diameter, with diffluent wall. The name is derived from the shape of the columellæ, which are mostly definitely pear-shaped, 200–300μ long. The spores are elliptical, 5–13μ by 4–8μ.

M. racemosus Fresenius is probably the most widely distributed of all the *Mucors*, and has frequently been redescribed under other names. It is found on almost every kind of damp material. Colonies are grey or brownish grey, of loose texture and normally less than 1 cm. high. The sporangiophores are simple at first, later becoming branched, with the branches irregularly placed and very unequal in length. Sporangia are globose, very unequal in size but mostly small, 20–70μ diameter, with walls which break in pieces when handled. The columellæ are spheric or oval with collarette (a portion of the broken sporangial wall left *in situ*) (Fig. 9), and the spores are mostly elliptical, 6–10μ by 5–8μ. The most characteristic feature of the species is the abundant production of chlamydospores, which are formed in aerial hyphæ, along the sporangiophores and even in the columellæ. They are of diverse shapes, colourless or yellow, smooth, and about 20μ in diameter (Fig. 11). *M. racemosus* can grow completely submerged in liquid media containing sugar, when it forms chains of yeast-like cells and, like yeast, produces alcohol.

M. spinosus van Tieghem (synonyms *M. spinescens* Lendner and *M. plumbeus* Bonorden) is of very common occurrence. Colonies are at first white, then dull grey and finally brownish grey, only a

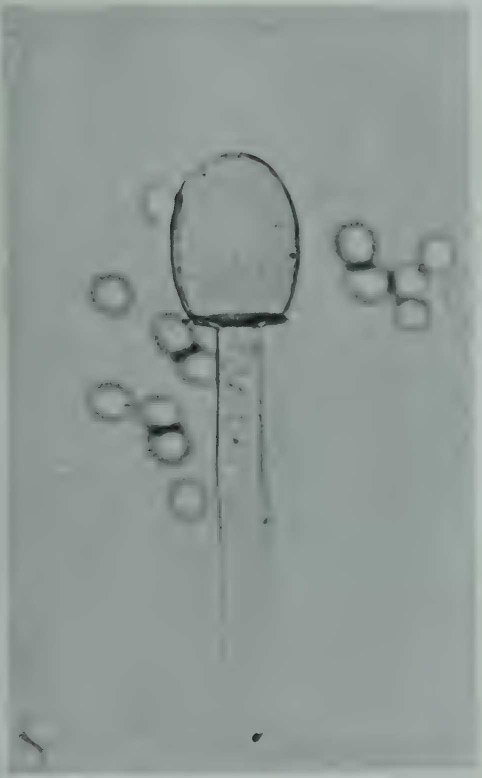


Fig. 9.—Oval columella with collarette—*Mucor racemosus.* × 500.

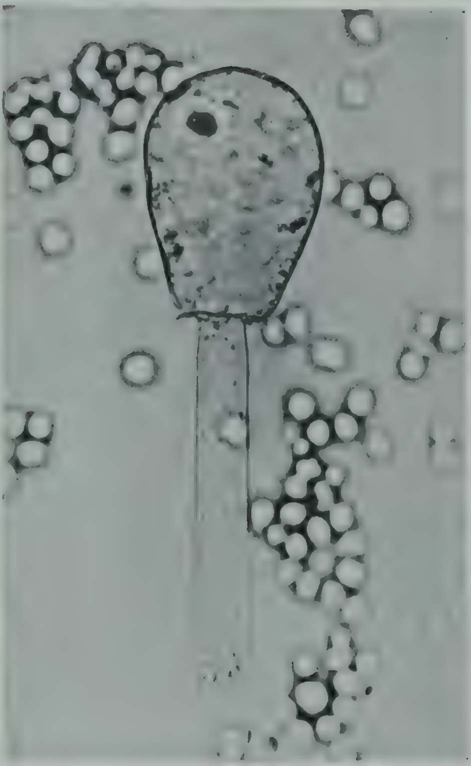


Fig. 10. Pyriform columella with small collarette. × 500.

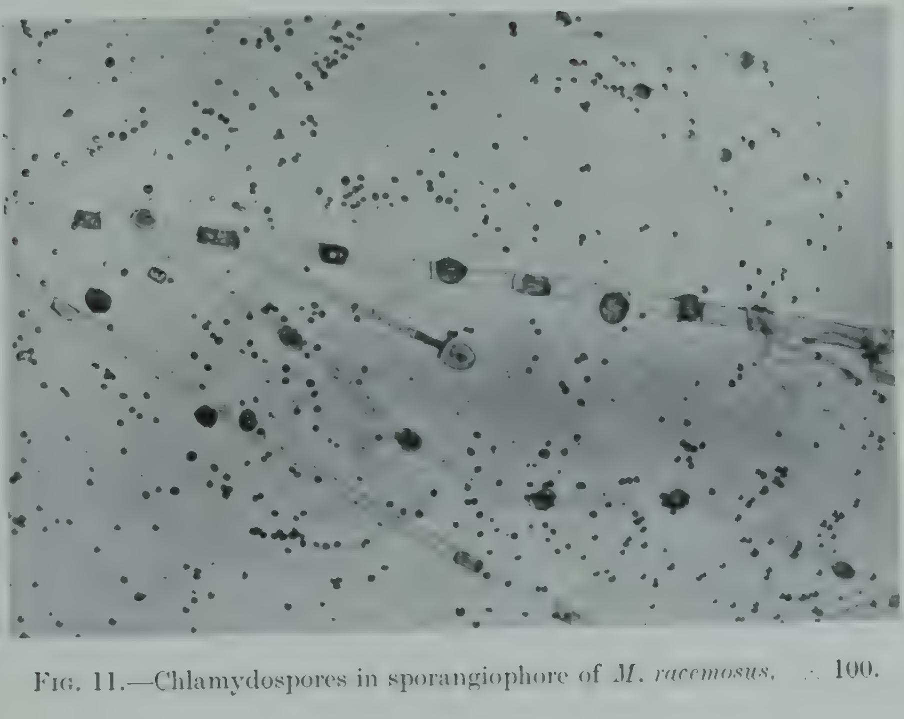


Fig. 11.—Chlamydospores in sporangiophore of *M. racemosus.* × 100.

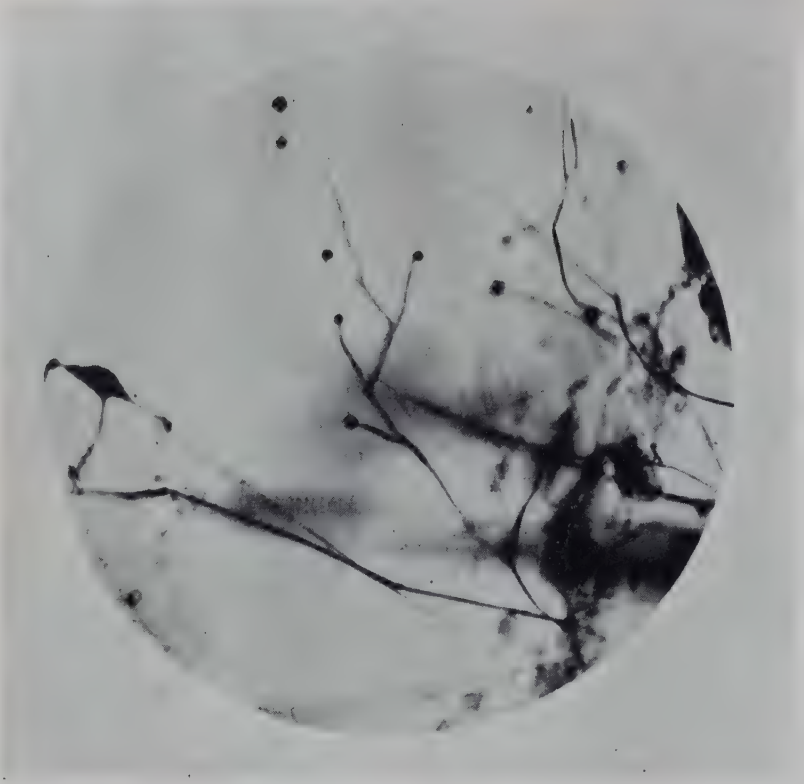


FIG. 12.—Sporangiophore of *M. spinosus* showing cymose branching, as seen in living culture (tube). × 50.

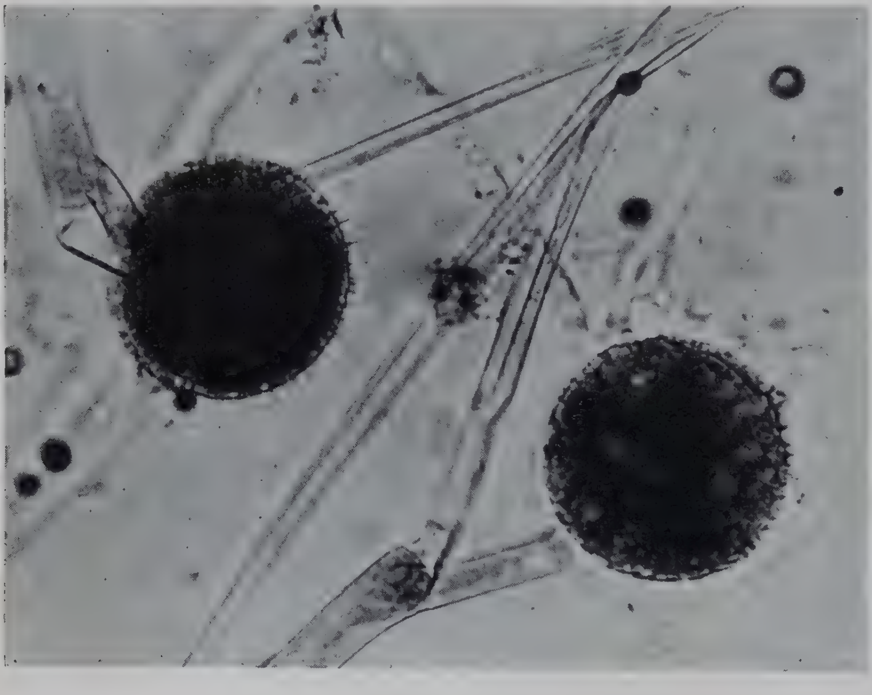


FIG. 13.—*M. spinosus*—young sporangia showing spinescent walls. × 500.

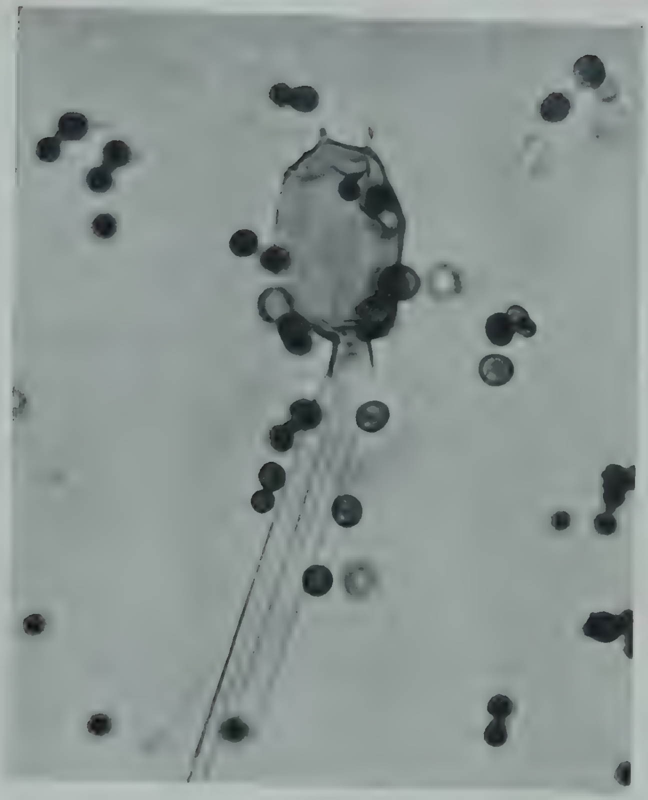


FIG. 14.—*M. spinosus*—columella with terminal spines. × 500.

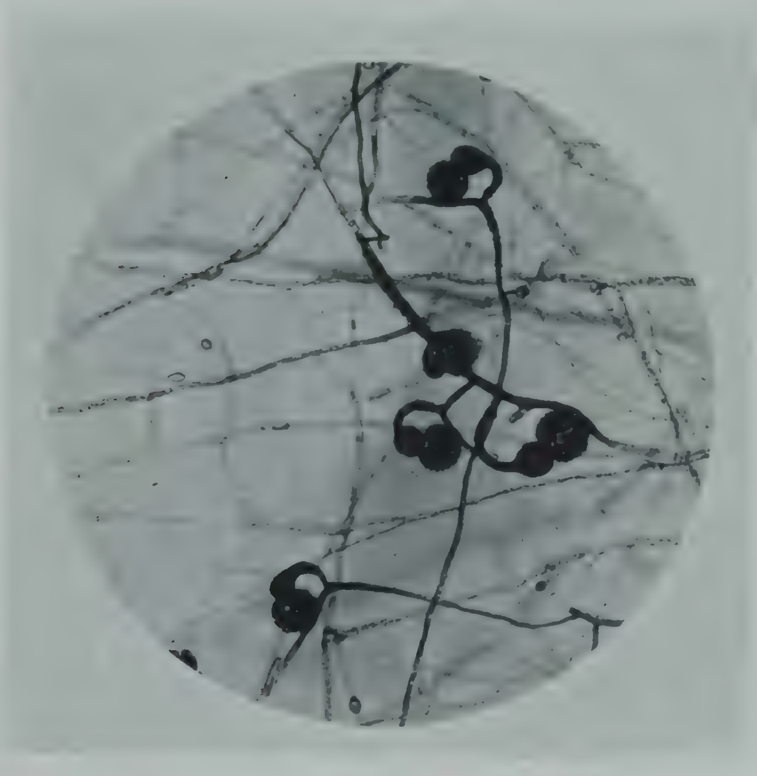


FIG. 15.—*Zygorhynchus Mœlleri*—zygospores (from tube culture). × 100.

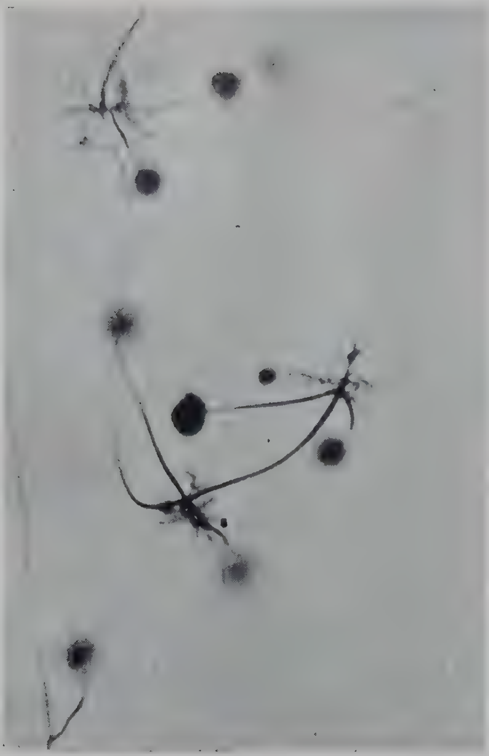


Fig. 16.—*Rhizopus nigricans* showing rhizoids and stolons (Petri dish culture). × 50.

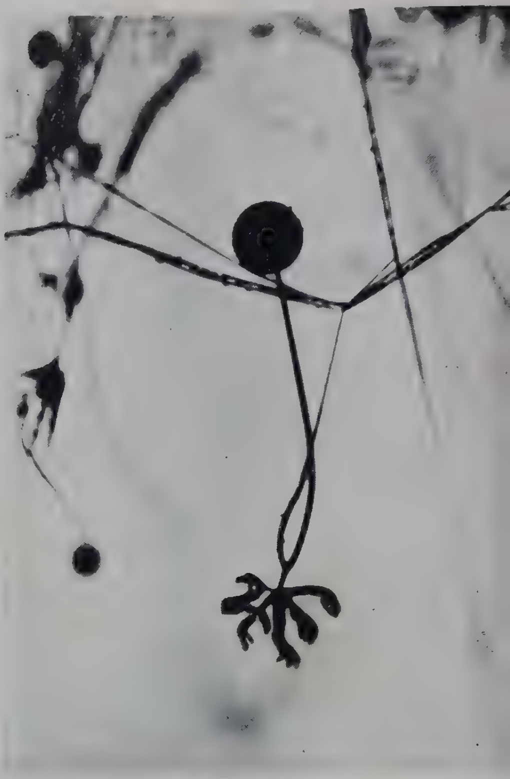


Fig. 17.—*Rh. nigricans*—rhizoids and sporangium (tube culture). × 50.

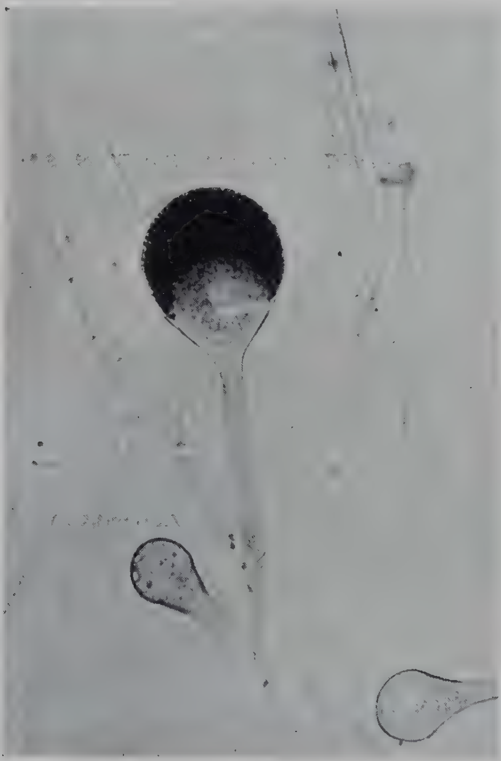


Fig. 18.—*Absidia ramosa*—sporangium, showing apophysis. × 250.

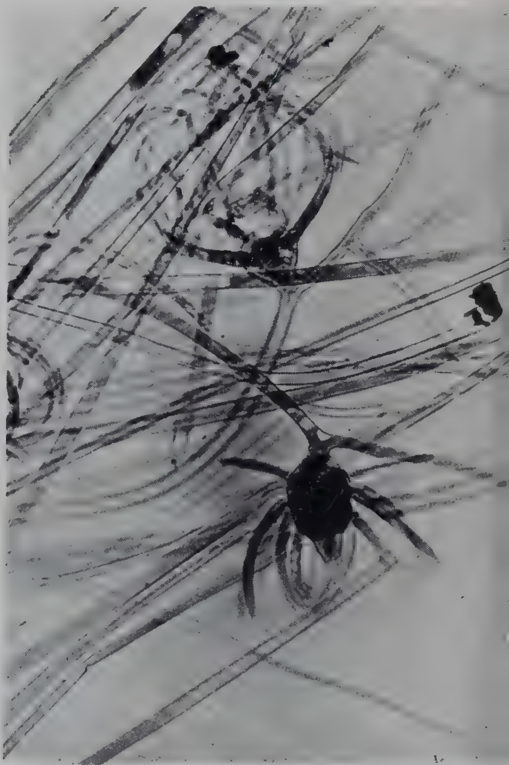


Fig. 19.—*Absidia spinosa*—formation of zygospores showing outgrowths from one suspensor. × 250.

ew millimetres high. Sporangiophores show sympodial branching (see Fig. 12) and are about 1 mm. long and 10μ diameter. Sporangia re nearly spherical, usually slightly flattened, very even in size, bout 65μ diameter, and are encrusted with fine needle-shaped rystals, thus appearing spinescent (Fig. 13). The columellæ are very haracteristic, being oval or pyriform with curious spiny projections t the top (Fig. 14). The spores are round, 7–8μ diameter, occasion-lly somewhat smaller.

M. Rouxii (Calmette) Wehmer was originally isolated from hinese rice by Calmette and named *Amylomyces Rouxii*. It was he first of a series of species of the Mucoraceæ to be used for the anufacture of alcohol by the "Amylo" process (Calmette, 1892; ollette and Boidin, 1897, 1898). It grows, but does not usually orm sporangia, on most ordinary media. On rice it grows normally, olonies being reddish in colour, with short, sparingly branched porangiophores, small sporangia 20–30μ diameter, elliptical spores bout 5μ long, and abundant chlamydospores, yellow to brown and p to 100μ in diameter.

M. hiemalis Wehmer is a common soil organism and hence is found n numerous soil-contaminated products. Colonies are yellowish or lear grey, 1–2 cm. high; sporangiophores simple or sympodially ranched; sporangia 50–80μ diameter, olive to greyish brown when ipe, with diffluent wall; columellæ spheric or oval with collarette, p to 50μ long; spores irregular in shape but mostly roughly oval, –9μ by 2·5–5μ, very characteristic, in spite of the variation in size, eing a fairly even mixture of large and small forms; chlamydo-pores formed in mycelium. The mould is heterothallic and both - and — strains are known.

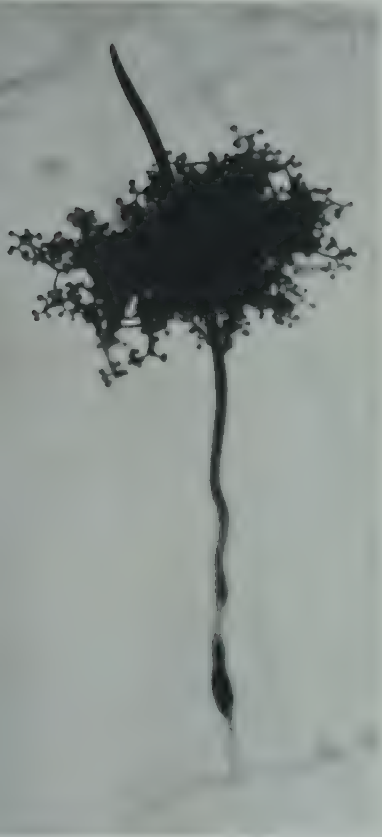
M. Ramannianus Möller is another fairly common soil organism. olonies are at first pinkish to brownish red, turning grey, only about mm. high; sporangiophores mostly unbranched, 2–6μ diameter; orangia reddish, small, 20–40μ diameter, with diffluent wall; lumellæ spheric, 5–10μ diameter; spores globose to short oval, -3μ long; chlamydospores numerous and of various shapes; giant lls formed in submerged mycelium.

Zygorhynchus is a small genus of moulds mostly inhabiting the il. The genus was not recognized by Lendner but is separated from *ucor* by most other authorities. All the species are homothallic d differ from *Mucor* in that the suspensors are very markedly equal in size (Fig. 15). Two species are of common occurrence in is country.

Z. Moelleri Vuillemin grows well on all media, forming a loose felt only a few millimetres high. Abundant zygospores are formed within two to three days, sporangiophores being more sparingly produced somewhat later. The sporangiophores are simple or irregularly branched, bearing grey sporangia which are mostly slightly broader than long, usually 48 by 50μ. Columellæ are definitely obovate, averaging 25 by 30μ, and the spores are oval, 4–5μ by 3μ. The zygospores are formed on bifurcated hyphæ, are spherical, 35–50μ, dark brown to black, and covered with short thorn-like projections.

Z. heterogamus Vuillemin is somewhat similar to the last species. Sporangiophores are branched, often more or less verticillate with two to four branches, sporangia spherical 50–60μ in diameter, columellæ spherical, and the spores round, 2–3μ in diameter. Zygospores are black, verrucose, varying in size from 45 to 150μ. Chlamydospores are formed in the mycelium.

Rhizopus. Species of this genus occur on all kinds of material and are common as aerial contaminants in the laboratory. On most culture media they grow with extreme rapidity, spreading widely by means of their stolons. They completely fill culture tubes and Petri dishes with dense cottony masses of mycelium, and can be a great nuisance from their habit of sporing along the line where the cover touches the edge of the dish, thus shedding spores outside. The *Rhizopi* are readily distinguished from *Mucors* by the presence of stolons (runners), often several centimetres long, and of tufts of root-like hyphæ (rhizoids) emerging from the points where the stolons touch the medium or the surface of the glass, also by their greyer colour and greater luxuriance of growth. Lendner (1908) describes 22 species, but some of these are rare, and a number of others are, except for the specialist, best regarded as strains of *R. nigricans*. *R. nigricans* Ehrenberg is of world-wide distribution and is found on all kinds of mouldy material. It is frequently the first mould to appear on stale bread and is often found on other foodstuffs in the home. Colonies spread very rapidly, completely filling tubes and Petri dishes in two to three days. Stolons are clearly differentiated, arising from and terminating in strong tufts of dark brown rhizoids (Figs. 16, 17). The sporangiophores are erect, arising in groups opposite the rhizoids, up to 2·5 mm. long and about 20μ in diameter bearing black, globose sporangia, up to 200μ in diameter. The spores are variously shaped, ovate, polygonal or angular, striated, and mostly 6–9μ in long axis.



IG. 20.—*Thamnidium elegans*—young sporangiophore with clusters of sporangioles (Petri dish culture). × 50.

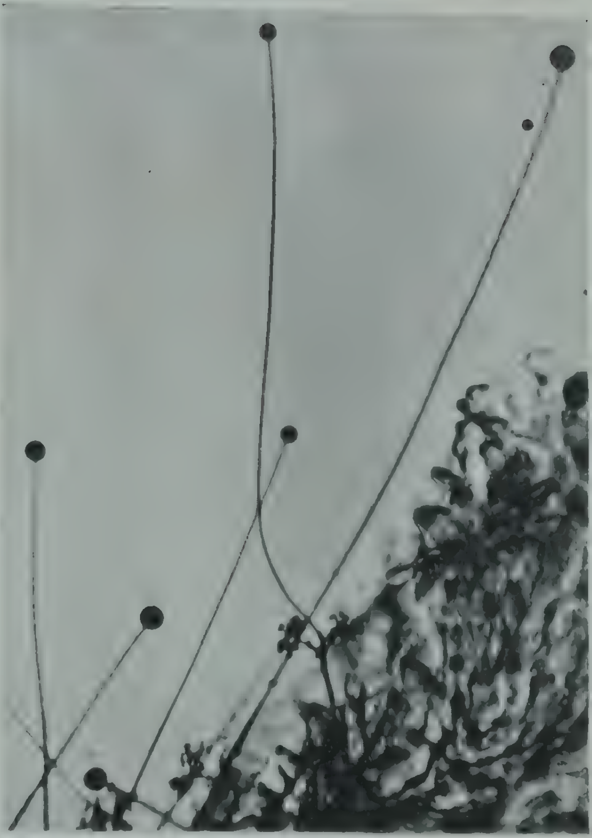


Fig. 21.—*Th. elegans*—mature sporangiophores with terminal sporangia (from culture on film of agar between two glass plates). × 25.

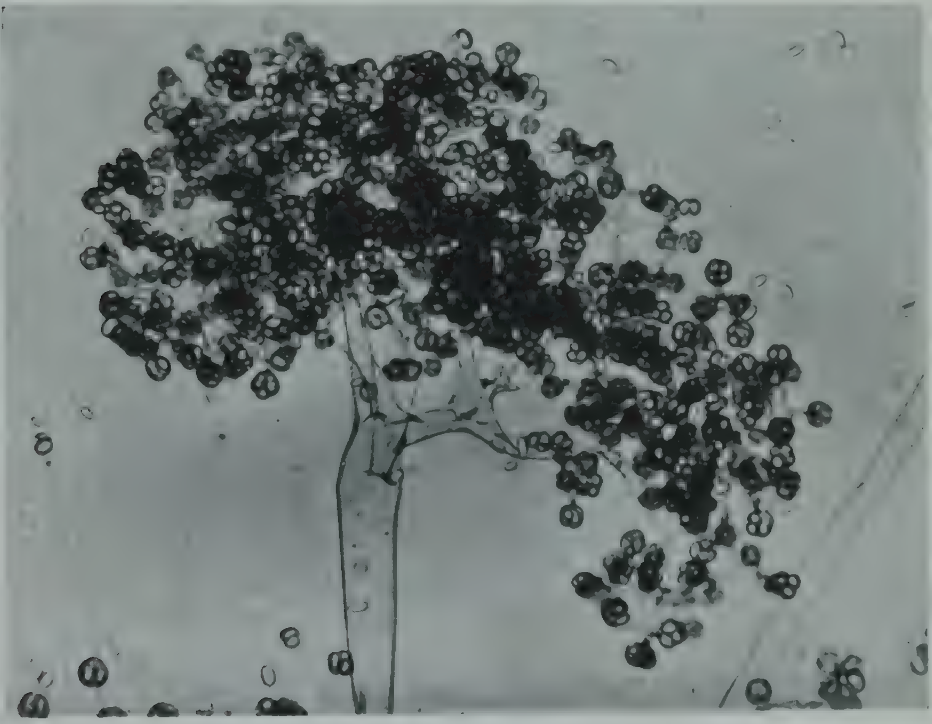


Fig. 22. *Th. elegans*—sporangioles. × 200.

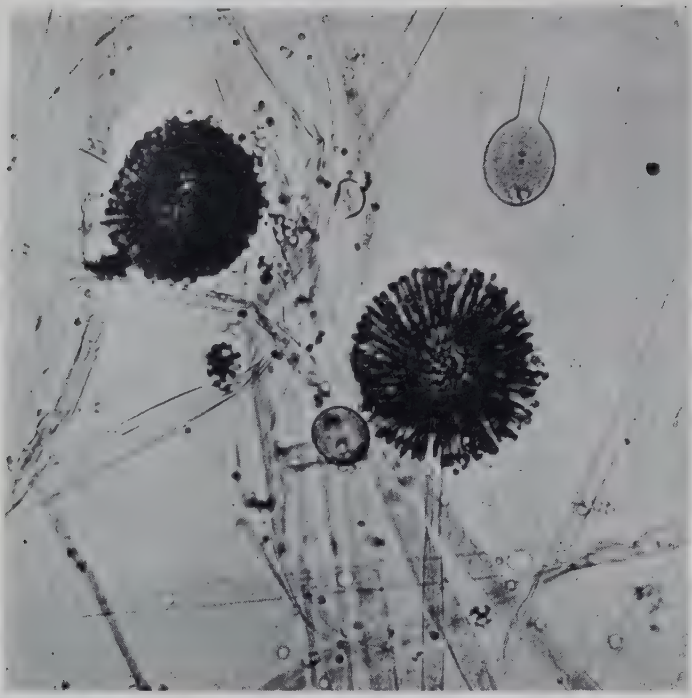


Fig. 23.—*Syncephalastrum racemosum*—spore heads. × 250. Note the resemblance to *Aspergillus.*

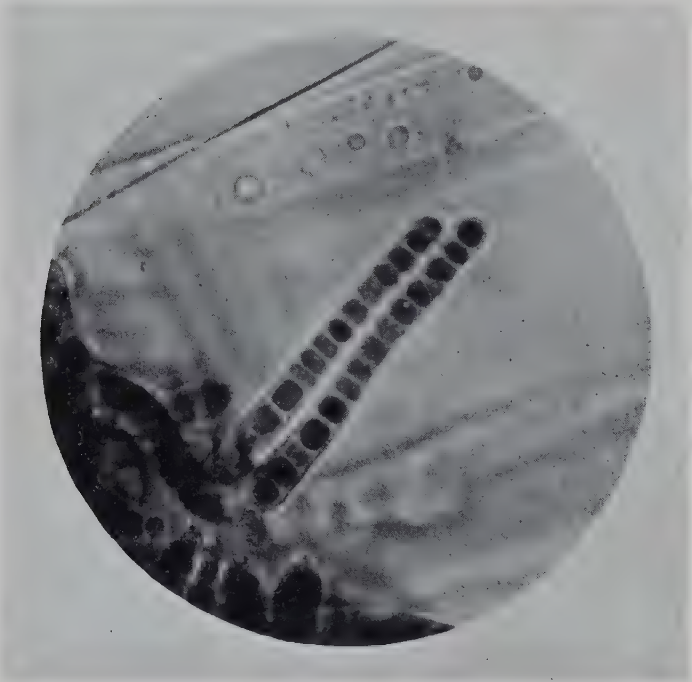


Fig. 24.—*S. racemosum*—tubular sporangia. × 1000.

R. arrhizus Fischer is distinguished from *R. nigricans* by its some-
hat less rampant growth and by the character of the rhizoids,
hich are pale in colour, short and ragged.

Two other species of *Rhizopus*, *R. japonicus* Vuillemin and *R.*
nkinensis Vuill., both of oriental origin, are of note because they
uccessively replaced *Mucor Rouxii* for the saccharification of starch
n the Amylo process of manufacturing alcohol. They are of no
nportance in other connections and are not likely to be met with
ordinary mycological work.

Absidia. Species of this genus differ in several respects from the
hizopi. The sporangiophores arise from the internodes of the
olons rather than from the points of attachment of the rhizoids,
e sporangia are small and pear-shaped, and probably the most
aracteristic feature of the genus is the well-marked "apophysis,"
e. a funnel-shaped base to the sporangium, where the walls of
orangium and columella are united (Fig. 18); the zygospores are
rrounded by coarse hairy outgrowths from one or both suspensors,
is feature serving to place at once the homothallic species of the
nus (Fig. 19). Zycha describes 17 species, and Naumov 29 species
d 2 varieties. Some of them are of common occurrence in the
il and appear sometimes in mixed cultures from mouldy material,
as aerial contaminants, but they are not found so frequently as are
ecies of *Mucor* and *Rhizopus* and are of little technical importance.

Phycomyces. Several species are known, but only one is com-
only encountered. *Ph. nitens* (Agardh) Kunze occurs mainly on
tty products and particularly in empty oil casks. It grows well
wort agar and some media made from plant extracts, but poorly
synthetic media. The sporangiophores are stiff, almost like wire,
k coloured and with a pronounced metallic sheen. In test-tube
tures they curl round in order to accommodate their length to
confines of the tube, but in the natural habitat of the species
y are approximately straight and up to about 30 cm. in length.
nitens is positively phototropic and, in the laboratory, can be
uced to form its characteristic long sporangiophores by growing
a rich medium, such as wort, contained at the bottom of a tall
sel, the sides of the latter being covered with some opaque
erial so that light can enter only from the top (Fig. 139).

hamnidium differs from the preceding genera in that the
rangiophores bear lateral clusters of ***sporangioles*** as well as large
ninal sporangia. The sporangioles resemble miniature sporangia,
taining from two to a dozen or more spores, and are formed on

richly branched outgrowths from near the base of the sporangiophor (Figs. 20, 21, 22).

Th. elegans Link is a fairly common mould and grows well o most culture media. It rapidly forms a granular, greyish growth few millimetres high, consisting chiefly of sporangioles. Later th sporangiophores extend to a length of 1 cm. or more before formin the large globose terminal sporangia, which are dark brown i colour and 150–250μ in diameter. The spores in the sporangiole are indistinguishable from those in the main sporangia. They ar ovate or bean-shaped, 8–12μ in long axis.

Syncephalastrum is a small genus of tropical moulds, but on species, *S. racemosum* (Cohn) Schröter (syn. *S. cinereum* Bainier is fairly often found on raw materials brought into this country. I grows luxuriantly in the laboratory, forming colonies resembling i texture those of a *Rhizopus*, with black heads produced on shor branches from trailing hyphæ. When mounted and viewed at low magnification the heads bear a striking resemblance to head of *Aspergillus* (Fig. 23). At a higher magnification the chains o spores are seen to be contained in long tubular sporangia whic radiate from the swollen end of the sporangiophore. Fig. 24, take at a high magnification, shows clearly that the spores are forme within, and distinct from the sporangial wall.

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CHAPTER IV

THE ASCOMYCETES

THE Ascomycetes constitute the largest of the classes of perfect Fungi, the number of species known being approximately 15,000. As may be expected in such a large group there is considerable diversity of form and structure. At one end of the scale are the unicellular organisms commonly known as yeasts, and, at the other, species with extensive mycelium and large and elaborate fruiting structures, such as the truffles.

The *ascus*, a structure which is peculiar to and which gives the name to the group, is a thin-walled receptacle enclosing the spores and usually rupturing at maturity. The ascus is distinguished from the sporangium of the Phycomycetes in the origin and method of formation of the spores, details of which are to be found in any work on Systematic Mycology. A more obvious difference is that he sporangium contains an indefinite number of spores, whilst in he great majority of Ascomycetes the ascus invariably contains 8 spores. In a few cases, which are of no importance here, the egular number is a multiple of 8, or, more strictly, a higher multiple of 2. In a few primitive members of the class, notably in *Endomyces*, he number is 4, whilst in the yeasts the number ranges from 1 to 8. n the simpler Ascomycetes the ascus is more or less globose, with he spores packed together, whilst the ***ascocarp***, i.e. the fruit-body containing the asci, is globose with the asci arranged irregularly or ntirely lacking, the asci then being produced either singly or in oose clusters. In the higher members the ascocarp takes definite orm, with club-shaped asci arranged in parallel series, often with ongated sterile cells (paraphyses) separating them. If the ascocarp globose or flask-shaped and closed at maturity it is called a ***rithecium***, if wide open at maturity an ***apothecium***. The primary assification of the Ascomycetes is based on the form of the ascocarp. any different classifications have been proposed, probably the most tisfactory being that of Gwynne-Vaughan and Barnes (1937).

scocarp, if present, either with no definite ostiole, or
shield-shaped, or with asci irregularly arranged . PLECTOMYCETES.
scocarp wide open at maturity, asci in parallel series . DISCOMYCETES.
scocarp flask-shaped when ripe, asci in parallel series PYRENOMYCETES.

The Discomycetes include about 4,000 species, very many of them parasitic, but some saprophytic in the soil or on dung. A number of the larger forms are fleshy and a few, such as the truffles and morels, edible.

The Pyrenomycetes form the largest of the three groups with about 10,000 species. Many common and serious plant diseases are caused by members of this group, but there are also a large number of saprophytic species and a very few which may be classed as moulds. The latter will be discussed below.

The Plectomycetes are divided into three orders, Plectascales, Erysiphales and Exoascales, of which the two latter include only obligate parasites and have asci arranged in parallel series like the Pyrenomycetes. The Plectascales include the great majority of the Ascomycetes which are of industrial importance.

Byssochlamys is of interest to the systematic mycologist as it produces clusters of 8-spored asci without any surrounding wall (peridium) and thus forms a link between the Endomycetaceæ, with solitary 4-spored asci, and the Gymnascaceæ and Aspergillaceæ with more or less definite perithecia containing 8-spored asci. In addition, *B. fulva* Olliver and Smith (1933) has assumed considerable importance in recent years as a cause of spoilage in canned and bottled fruits. The mature ascospores can withstand a much higher temperature than can the spores of most fungi and sometimes survive the commercial sterilizing process. The fungus has also the power of growing in an atmosphere containing very little oxygen, or even completely submerged in liquid, where the only oxygen available is the small amount dissolved. Spoiled fruit frequently shows no visible colonies of the mould and the cans are not blown as when the spoilage is due to anaerobic bacteria, the only evidence of the presence of the fungus being a general softening, or sometimes complete disintegration, of the fruit, such as would occur if cooking had been unduly prolonged.

The mould grows well on all ordinary media, with colonies of loose cottony texture, becoming fulvous (tan to sandy brown) as spores develop. The conidial fructification is of the *Pœcilomyces* type (see Chapter IX) with long chains of ovate spores, 4–9μ by 2·3–2·5μ (Fig. 25). The asci develop rapidly in compact clusters, without any trace of peridium (Fig. 26), each ascus containing 8 ovate spores, 6–6·5μ by 4·3–4·5μ. *B. nivea* Westling is similar, but the colonies are white, only turning slightly brown in age, and the ascospores are somewhat smaller than those of *B. fulva*.

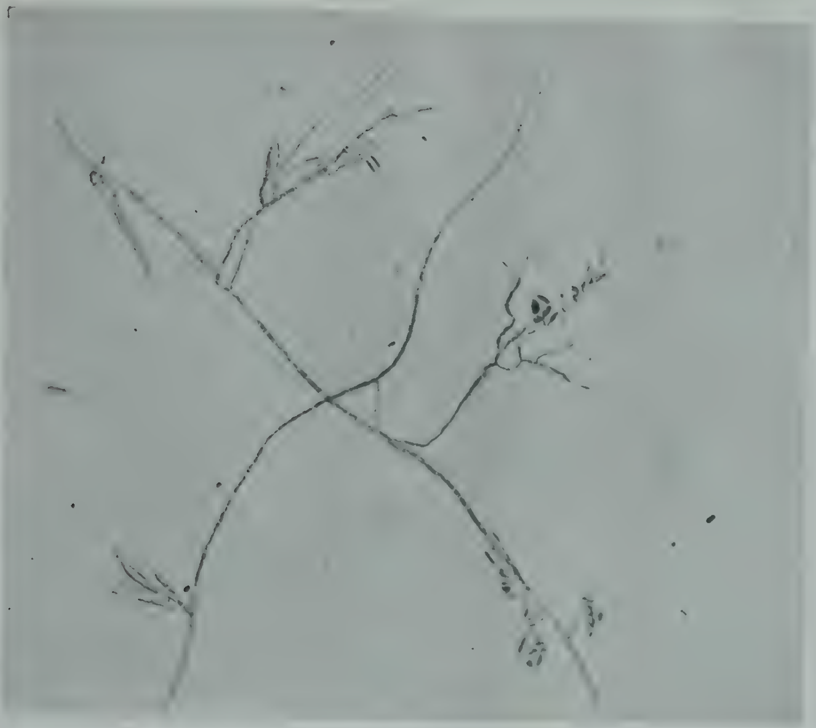

FIG. 25.—*Byssochlamys fulva*—conidiophores (from slide culture). × 250.

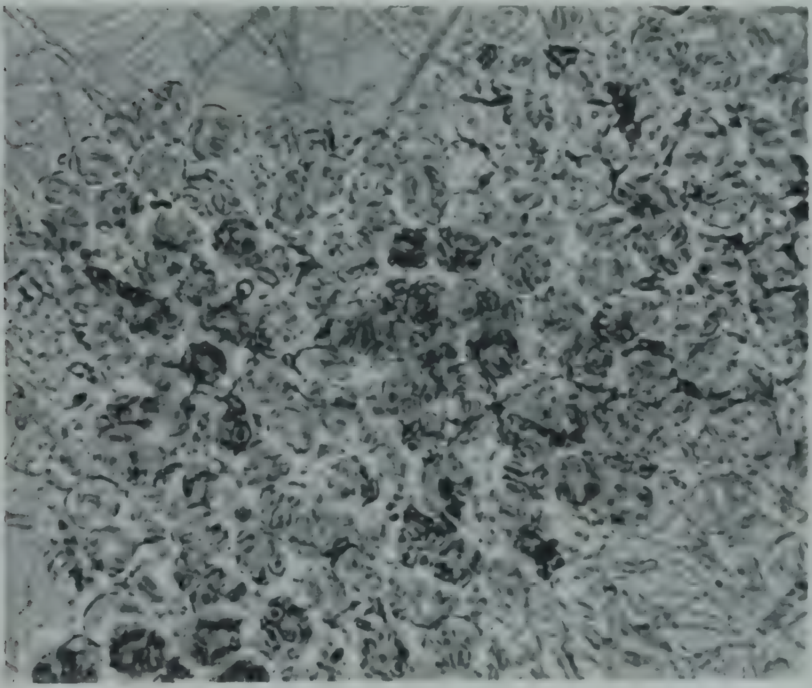

FIG. 26.—*B. fulva*—cluster of asci. × 500.

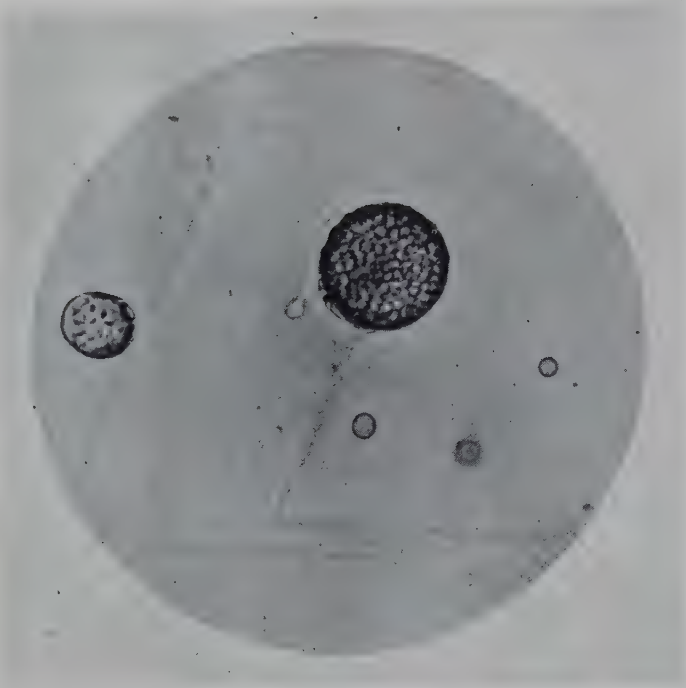

Fig. 27.—*Momascus purpureus*—stalked perithecium. × 250.

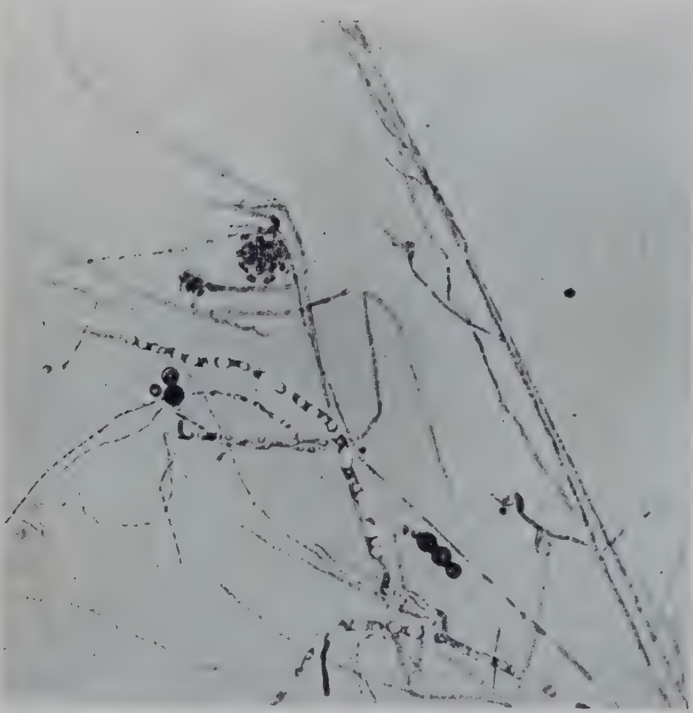

Fig. 28.—*M. purpureus*—young perithecium and conidia. × 250.

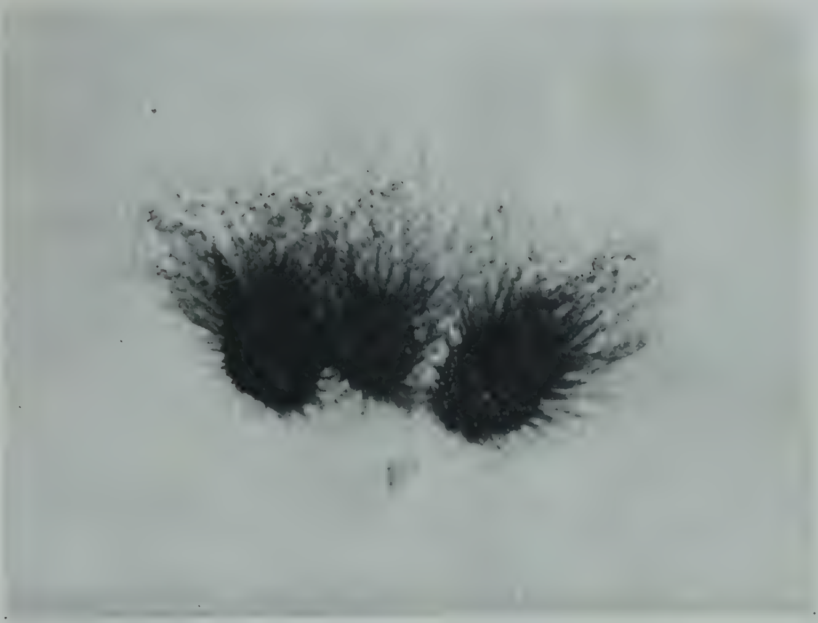

FIG. 29.—*Chætomium globosum*—perithecia. × 50.

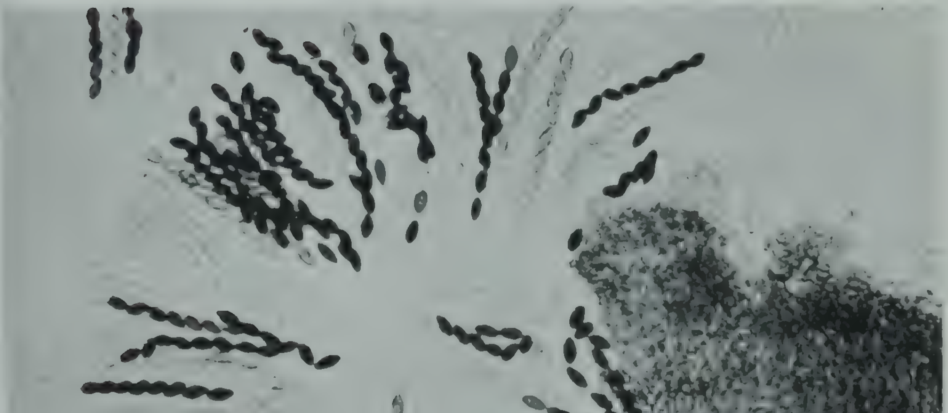

FIG. 30.—*Neurospora sitophila*—crushed perithecium. × 100.

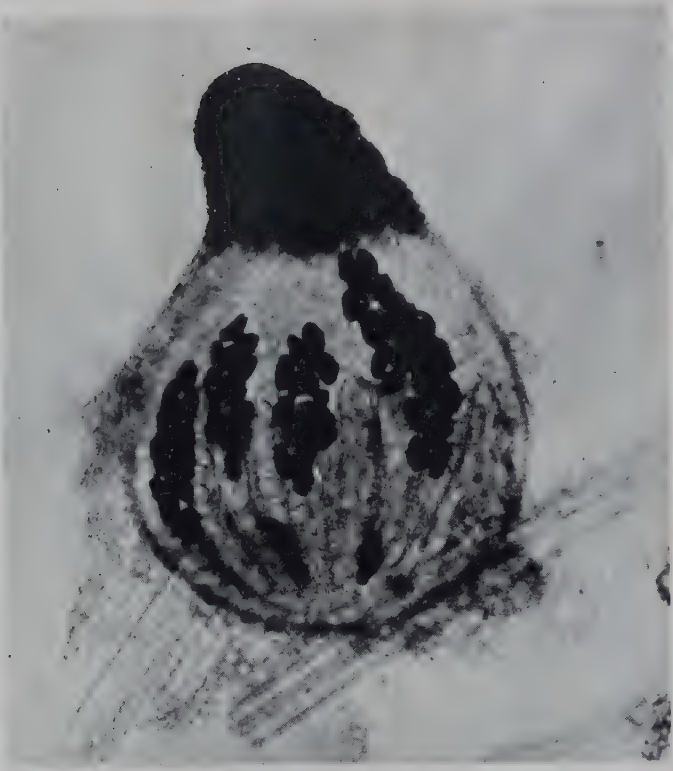

Fig. 31.—*Sordaria sp.*—perithecium in optical section. × 100.

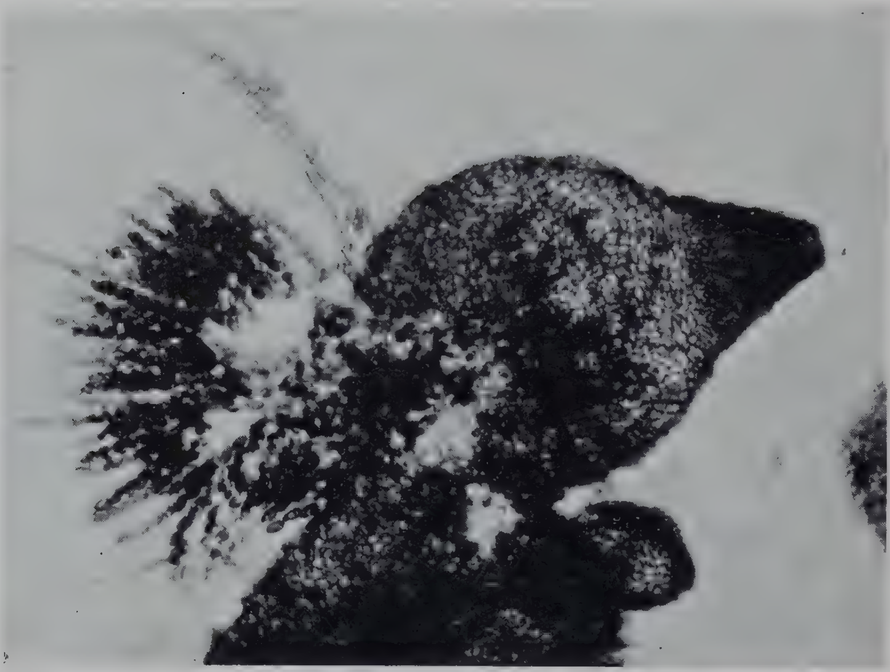

Fig. 32.—Typical Pyrenomycete, *Melanospora destruens*—crushed perithecium. × 100.

Eurotium is characterized by the production of globose perithecia, mostly bright yellow, without any neck or ostiole, breaking irregularly at maturity to liberate the spores. Asci are globose or ovate, very thin-walled, 8-spored, arranged irregularly in the perithecium, and the ascospores are more or less lenticular, splitting along a median longitudinal furrow at germination. The best-known species of the genus are more commonly known as the *Aspergillus glaucus* series. The conidial and ascosporic phases were long thought to be separate and distinct species until De Bary in 1854 showed their real relationship. Further details are given in the chapter on Aspergillus (Chapter VIII).

Monascus. M. purpureus Went is not uncommon, particularly in dairy products, and is the organism which gives the characteristic colour to Chinese red rice. The perithecia are produced singly on stalks (Fig. 27), whilst the ovate conidia are formed in short chains (Fig. 28). The generic name was bestowed at a time when the perithecium was thought to constitute a single, many-spored ascus. It is now known that normal asci are produced, but these break up rapidly at maturity, leaving the somewhat thin-walled perithecium full of loose spores. Colonies form a rather thin, spreading growth, of a pronounced reddish or purple colour.

Chætomium is probably the only genus of Pyrenomycetes at all commonly encountered in industrial work. A number of species are known, all characterized by black perithecia with short necks and beset with long stiff hairs, which are variously straight, branched or curled. They are found chiefly on cellulosic materials and thrive particularly on paper. When kept in culture a strip of filter paper, only partly immersed in an ordinary culture medium, usually ensures satisfactory production of perithecia. *C. globosum* Kunze is the most commonly occurring species. It grows vigorously in culture, the colonies being brownish yellow, the perithecial hairs spirally coiled (Fig. 29) and the spores brownish, elliptical with more or less pointed ends, in size about 9 × 7μ. There is an excellent monograph of the genus by Chivers (1915), with descriptions and illustrations of 28 species of *Chætomium* and 2 of the related genus *Ascotricha*. *C. chartarum* Berk., a species frequently mentioned but almost invariably misinterpreted in technical literature, is shown to belong to *Ascotricha*.

Neurospora is the name given to the perfect stage of the red bread-mould, *Monilia sitophila* (Montagne) Saccardo. Since the fungus is heterothallic, perithecia are obtained only when the mycelia of two strains of opposite sex come in contact (Shear and Dodge, 1927). (See Fig. 30.)

A number of other Ascomycetes, mostly plant parasites or saprophytes on plant residues, strayed from their natural habitat, are occasionally encountered in mixed cultures. Many of them are sterile under ordinary laboratory conditions and cannot be recognized unless previously obtained from a known source, but a few, such as *Sordaria*, *Pleospora* and *Melanospora*, frequently grow well in artificial culture and produce normal perithecia. *Sordaria* (Fig. 31) has pale coloured perithecia, with short darker neck, and the spores usually have a mucous sheath. *Pleospora* has muriform ascospores, and often produces conidia of similar appearance, the imperfect stages of the genus being *Alternaria* and *Macrosporium*. *Melanospora* is a somewhat badly defined genus, but one species, *M. destruens* Shear (Fig. 32), occurs fairly often as isolated colonies in Petri dish cultures. It grows extremely rapidly on wort agar and tends to swamp everything else. Colonies are dark coloured, with brown perithecia which appear almost black in mass. The spores are dark brown, ovate to biconvex, 17–24 × 11–13μ, produced in single rows in long cylindrical asci. Dark coloured perithecia with walls which are opaque under the microscope can be distinguished from pycnidia of similar gross appearance by gently crushing them under a cover-glass, when it is nearly always possible to find immature asci containing the normal 8 spores. Such species are of no particular importance outside the province of plant pathology.

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CHAPTER V

THE YEASTS AND RELATED FUNGI

The fungi known as yeasts are organisms of very great economic mportance. Certain species are used in all parts of the world for he process of baking and for the production of alcoholic beverages y fermentation, for they secrete enzymes which convert sugars into lcohol and carbon dioxide. Others are responsible for the developnent of special flavours in certain wines, after the main fermentation s completed. Still others are encountered as contaminants in the ermentation industries, where their presence is undesirable because hey consume alcohol or produce unpleasant flavours. Some of the easts, but more often members of a group of organisms of similar imple structure, usually classed as the non-sporing yeasts, are ommon accompaniments of moulds, frequently appearing in large umbers when mouldy products are plated out.

General Morphology. Young colonies of these organisms, oth true yeasts and non-sporing yeasts, when growing on solid edia have almost always a very characteristic appearance, being oist and somewhat slimy. The colour is usually whitish, cream r pink, but a few species are otherwise coloured. In some species olonies change little with age, but others gradually become wrinkled nd dryer in appearance. (It should be noted that some common prophytic bacteria grow well on the usual mycological culture edia and produce colonies which may readily be mistaken for those yeasts. These may, of course, be distinguished by microscopical amination.)

The majority of the numerous species grow as loose aggregates single cells, which may be globose, ovoid, pyriform, or more or ss elongated. When growing very vigorously they often form ains of cells, loosely attached, somewhat resembling mycelium, t most species do not form true mycelium. There is, however, sharp line of demarcation between the unicellular yeasts and ulticellular fungi which do form true mycelium. The normal ethod of vegetative propagation in all but a few of the industrially portant species is by budding, and microscopical examination of ecimens mounted in fluid, or fixed and stained, usually reveals all ges of this characteristic process (Figs. 33, 36, 37). When about to reproduce, the mature cell puts forth a nipple-like projection; as this grows a constriction develops between the future daughter cell and the parent; after a period of further growth, in which the projection rounds up to approximately the same shape as the parent cell, the constriction deepens and the new cell becomes detached. A single cell may produce one, two, or several buds. In the process designated "bipolar budding" budding takes place only at one or both ends of an ellipsoidal or elongate cell (Fig. 37), whilst in "multipolar budding" one to several buds are produced and these are not formed at any fixed points. A different process is found in the genus *Schizosaccharomyces*, and less regularly in some other genera, in which vegetative propagation is by binary fission, each mature cell dividing transversely into two approximately equal parts (Fig. 34).

As indicated above, these organisms, which are often collectively referred to as yeasts, are divided into two main groups, the true yeasts (also known as the sporing, or sporogenous yeasts) and the non-sporing (asporogenous) yeasts. The first group includes only those species which are known to produce ascospores and are, therefore, Ascomycetes, closely related to the simpler Plectomycetes. The asporogenous yeasts were for long regarded as imperfect forms, related to the sporogenous yeasts in the same way as the Fungi Imperfecti are related to the filamentous Ascomycetes. It is now known that, whilst this relationship undoubtedly holds for many species of the group, there are a number of other species which are closely related to the Basidiomycetes.

THE TRUE YEASTS

The modes of production of ascospores have been much studied and are important diagnostic characters in all schemes of classification. In a broad sense it may be taken that any vegetative cell may, under suitable conditions, become an ascus, distinguished from a normal vegetative cell by the fact that it is an envelope containing one or more spores, each spore with its own definite limiting membrane. The number of spores in the ascus may be any number from one to four, or, in a few species, eight.

Asci may be produced in three ways: by conjugation of two equal cells—isogamic conjugation; by conjugation of unequal cells—heterogamic conjugation; or from single cells without any trace of conjugation—parthenogenesis. Recent work has shown, however, that most, if not all, of the so-called parthenogenetic species exhibit sexuality, in that the ascospores, or small daughter cells formed by the ascospores, fuse in pairs to give normal cells. It has been found possible, in some cases, to separate single ascospores and to bring about the fusion of spores from different races of yeasts. The cells resulting from the fusion propagate to give normal colonies, and the production of hybrids by this method already shows promise of giving new yeasts of commercial value. A useful summary of recent work in this connection is given by Henrici (1941).

The taxonomy of the yeasts is difficult and only a brief outline can be given here. Fuller treatments are to be found in the monographs of Guilliermond (1912, 1920) and Stelling-Dekker (1931), and in reviews by Guilliermond (1940) and Henrici (1941).

The most generally accepted classification is that of Stelling-Dekker. She includes in one family, the Endomycetaceæ, all the ascospore-forming yeasts and also a number of fungi which form true mycelium but which resemble the yeasts in producing naked asci. The family is divided into four sub-families, of which the salient features are as follows:

Sub-family 1—EREMASCOIDEÆ. Vegetative growth entirely mycelial; ascospores hat-shaped; asci formed by isogamic conjugation. There is but one genus, *Eremascus*, and this is of academic interest only.

Sub-family 2—ENDOMYCOIDEÆ. Growth form either mycelium with oidia or oidia alone, multiplying by transverse fission. There are two genera, *Endomyces*, forming mycelium and with 4 round or hat-shaped ascospores, and *Schizosaccharomyces*, with no mycelium and with 4 or 8 round spores.

Sub-family 3—SACCHAROMYCOIDEÆ. Growth form either myce-ium with conidial buds and occasionally oidia, or budding yeast ells; multiplication by transverse fission or by budding; spores of arious shapes; asci formed by isogamic or heterogamic conjugation r parthonogenetically. This, the largest of the sub-families, is urther divided into 3 tribes.

Tribe A—*Endomycopseæ*. Growth form mycelium with conidial buds and occasionally oidia, multiplying by transverse fission and by multipolar budding; ascospores hat-shaped, sickle-shaped or ridged. There is only one genus, *Endomycopsis*, which includes a number of species originally placed in *Endomyces*.

Tribe B—*Saccharomyceteæ*. Growth form yeast cells with no mycelium, multiplying by multipolar budding; ascospores variously shaped. This tribe includes the majority of the yeasts which are of commercial value. There are 6 genera, *Saccharomyces*, *Pichia*, *Hansenula*, *Torulaspora*, *Debaryomyces* and *Schwanniomyces*.

Tribe C—*Nadsonieæ*. Growth form yeast cells with no mycelium, multiplying by bipolar budding. There are 3 genera, *Saccharomycodes*, with ascospores which regularly conjugate, *Hanseniaspora*, with asci formed parthenogenetically, and *Nadsonia*, in which the spores are produced in a bud from a cell which is itself the product of heterogamic conjugation.

Sub-family 4—NEMATOSPOROIDEÆ. Growth form mycelium and budding yeast cells; ascospores needle-shaped or spindle-shaped, with or without flagella. There are 3 genera, *Monosporella*, *Nematospora* and *Coccidiascus*, none of which is important.

Of the 16 genera recognized by Stelling-Dekker several contain no industrially important species and will not be considered here. Some of the species have never even been cultivated, whilst others have so far been studied only from the systematic point of view. The descriptions given below of the important genera and species are necessarily brief, and the reader is referred to the monographs already cited, and to the literature of the fermentation industries, for further information.

Endomycopsis. Only one species, *E. vernalis* (Ludwig) Dekker, is of importance. It has been much studied as a means of producing fat from carbohydrates (see Chapter XIV). On wort agar colonies are greyish, of somewhat tough consistency, much folded and wrinkled; mycelium in short lengths, up to about 60μ, branched, breaking up into oidia and forming conidial buds; asci not often produced.

Schizosaccharomyces. Two species are recognized. *S. octosporus* Beijerinck has been found on various kinds of fruit from Greece and Asia Minor. Grown in wort the cells are oval to cylindrical, 6–12 × 4–5μ. Asci are readily formed and contain 8 round spores, about 4μ in diameter. *S. Pombe* Lindner was isolated from African millet beer. Vegetative cells are cylindrical with rounded ends, 6–15 × 3–4μ (Fig. 34), and asci contain 1–4 spores, round and smooth, 2–2·5μ in diameter. This species has been shown by Underkofler, McPherson and Fulmer (1937) to give very good yields of alcohol by fermentation of an unhydrolysed syrup prepared from Jerusalem artichokes.

Saccharomyces. This genus includes most of the yeasts which are used for the production of alcoholic liquors. Vegetative cells

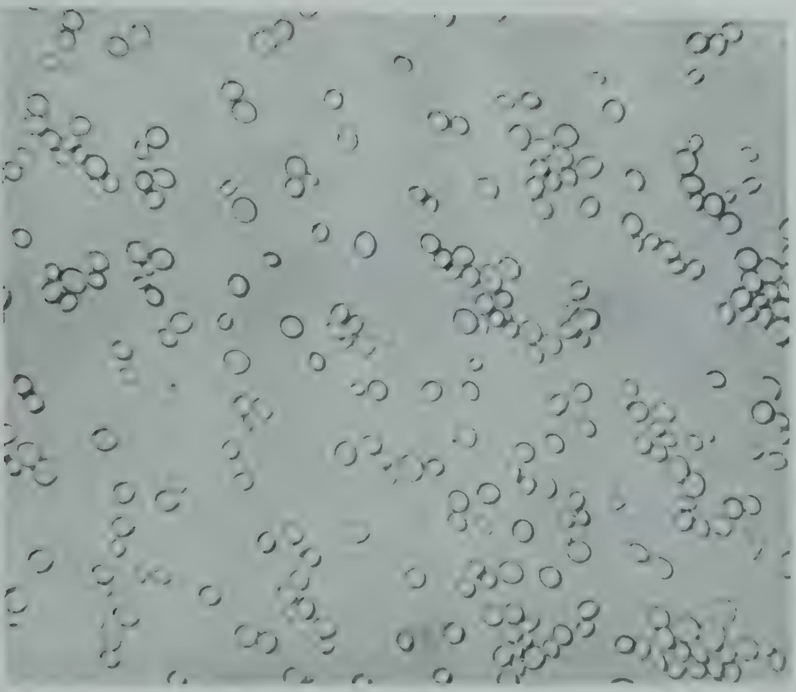

FIG. 33.—*Saccharomyces cerevisiæ*—vegetative cells, some in various stages of budding. × 500.

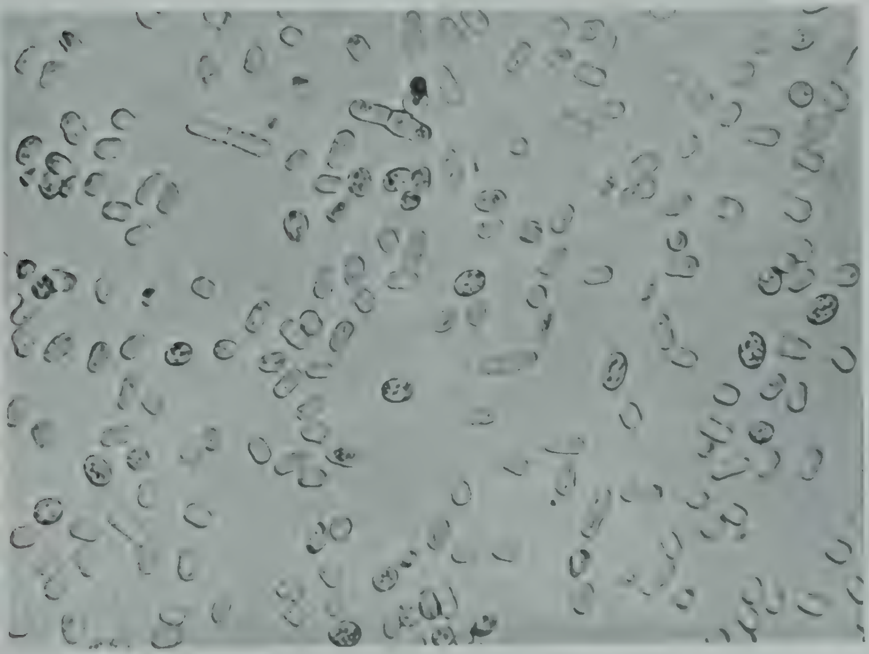

FIG. 34.—*Schizosaccharomyces Pombe*—vegetative cells propagating by binary fission. × 500.

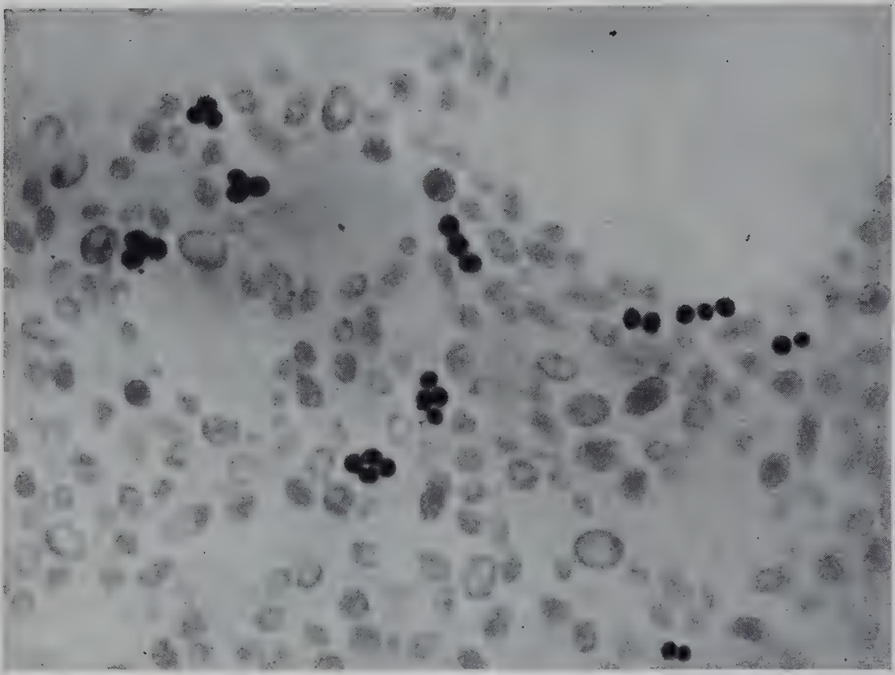

FIG. 35.—*Saccharomyces sp.*—ascospores, stained film. × 1000.

re round, ovate or elongated; no dry pellicle is formed on wort; scospores are round, kidney-shaped or hat-shaped, 1–4 per ascus, roduced parthenogenetically (sub-genus *Saccharomyces sensu stricto*) r sometimes sexually (sub-genus *Zygosaccharomyces*); all species erment glucose, lævulose and mannose, and usually other sugars in ddition. Stelling-Dekker recognizes 24 species and 12 varieties of *accharomyces* and 17 spp. and 1 var. of *Zygosaccharomyces.* Keys o the genus are based largely on fermentation reactions, ability to tilize nitrates as source of nitrogen and ability to grow on media ith alcohol as source of carbon. In general, any species which will erment any other sugar can ferment glucose, lævulose and mannose Kluyver's laws). In the diagnoses given below, therefore, these hree hexoses are omitted from the lists of sugars fermented. Ability o ferment raffinose 1/3 signifies that this trisaccharide is hydrolysed o lævulose and melibiose and only the former fermented.

S. cerevisiæ Hansen. This species is the typical "top-yeast" of he brewing industry (see below) and is also grown on the large cale for use in baking. Colonies on wort agar are soft and moist, ream coloured; cells, in wort, round, ovate or pyriform, in young ultures 4–14 × 3–7μ, with a length/breadth ratio varying from 1 : 1 2 : 1; elongated cells produced in old cultures, often in chains; wort a sediment, often a ring and occasionally a thin scum; scospores round and smooth; galactose, saccharose, maltose and ffinose 1/3 fermented; nitrates not utilized; growth on alcohol edium fairly good but atypical.

S. cerevisiæ var. *ellipsoideus* (Hansen) Dekker, commonly referred as *S. ellipsoideus*, is the species used for the fermentation of wine. s occurrence on grapes and in the soil of vineyards accounts for e success of vinous fermentation during countless ages before the ture of the process was understood. At the present time pure lture methods are used to some extent in the wine industry and number of strains with special trade names are in regular use for e production of different classes of wine.

This variety differs from *S. cerevisiæ* in the shape of the cells, ich are seldom round but mostly long oval or pyriform, with a ratio of 2 : 1, and in its higher tolerance to alcohol.

S. carlsbergensis Hansen is the typical "bottom-yeast" used in ewing. Cells ovate, in young culture 7–10 × 3–5μ, mostly detached; duction of spore formation difficult; in wort a sediment and tardily light scum; ferments galactose, saccharose, maltose and raffinose mpletely; nitrate not utilized; poor growth on alcohol medium.

BREWING YEASTS. A number of strains with trade names have been regularly propagated since the introduction, by Hansen, o pure culture methods into the brewing industry. There are two main groups of these, known respectively as "top-yeasts" and "bottom-yeasts." The top-yeasts produce CO_2 vigorously and the cells have a tendency to become attached in clusters. They are carried to the surface during the fermentation by the rising gas and eventually form a scum. These strains are used for brewing the typically English beers and ales. Bottom-yeasts are used for the preparation of the light Continental beers typified by the well-known "lager." In these a prolonged fermentation is carried out at a comparatively low temperature and the yeast cells stay at the bottom of the liquor. Distillers use top yeasts of high activity and carry out the fermentation at a high temperature in order to obtain rapid production of alcohol in maximum yields.

BAKERS' YEAST. The pressed yeast used in bakeries was at one time produced by breweries and distilleries, the mass of cells formed during the fermentation being separated by centrifuging, then washed and pressed for the market. Distillery yeast is more suitable for baking than is brewery yeast, since the latter is dark in colour and somewhat bitter, due to the presence of extractive matter from the hops which are added to the wort prior to the fermentation. At the present time most of the yeast used in baking is manufactured specially for the purpose. In the manufacture of potable liquors the aim is to utilize to the full the biochemical activities of the yeast cells, with maximum production of alcohol and CO_2, and to keep down undue multiplication of the cells, which would result in loss of valuable fermentable material. In the manufacture of compressed yeast, on the other hand, the aim is to obtain maximum growth and minimum production of alcohol. This is achieved by vigorous aeration of the culture medium and by addition of nutrients in stages during the process, to keep pace with the increase in the number of cells. Full details of the manufacturing process are given by Walter (1940).

S. pastorianus Hansen is best known for its production of unpleasant flavours in beer, in which it occurs as a contaminant. Cells ovate or, more usually, elongated, commonly described as sausage-shaped, 4–15 × 3–7μ, often forming long chains; ascospores roun and smooth; in wort a sediment and soon a scum; ferments galactose saccharose, maltose and raffinose completely; nitrates not utilized poor growth on alcohol medium.

S. fragilis Jörgensen (syn. *S. Kefir*) is used for the preparation of
ermented liquors from milk, such as Kefir and Koumyss. It is a
ottom yeast capable of fermenting lactose and producing therefrom
uch CO_2 and comparatively little alcohol. In the countries where
hese and similar milk products are made, pure culture methods are
ot used and the actual agent of fermentation is a mixture of various
easts and bacteria, in which, however, *S. fragilis* predominates.
ells ovate to elongate, 6–10 × 2·5–5·5μ, often forming chains;
rments galactose, saccharose, lactose and raffinose 1/3; nitrates
ot utilized; fairly good growth on alcohol medium.

Other species and strains of the genus *Saccharomyces* are respon-
ble for the alcoholic fermentation of cider and perry. The wild
easts which occur on the fruit are still relied on almost exclusively,
nd usually a mixture of several species is present during the fermen-
tion. In the East, alcoholic liquors are made chiefly from rice and,
hilst yeasts usually take some part in the fermentation, the main
gents are moulds.

Zygosaccharomyces. Several species of this sub-genus are of
ecial interest owing to their ability to grow on, and ferment,
aterials containing very high percentages of sugar. They have
een studied chiefly in connection with the fermentation, and
nsequent spoilage, of honey. A useful summary of present-day
nowledge of these so-called "osmophilic yeasts" is given in
enrici's review (loc. cit.).

***Pichia* and *Hansenula*.** Both genera are best known in this
untry as contaminants of alcoholic liquors. They grow as dry,
rinkled pellicles on the surface of the liquid and their metabolism
sugars is more oxidative than fermentative. If fermentation takes
ace the main products are esters and not alcohol. Most species
n utilize alcohol as a source of carbon and hence can constitute
serious nuisance in the fermentation industries. Species of both
nera also occur in the "flor" of sherry and of certain wines of
Arbois district of France, this being a film of yeasts which is
ponsible for the development of the special flavours of these
nes. A paper by Hohl and Cruess (1940) gives full information
cerning these "flor" yeasts.

Species of *Pichia* have cells of various shapes with a strong
dency to the formation of pseudomycelium; ascospores are mostly
mispherical, more rarely hat-shaped or angular; fermentation
sugars nil or slight. *Hansenula* (formerly *Willia*) has hat-shaped
ores and ferments strongly.

Hanseniaspora. The vegetative cells are ovate or lemon-shaped multiplying by bipolar budding, hence the common name "apiculate yeasts" (see Fig. 37). Species of this genus (and of *Klœckera*, a closely related genus of non-sporing yeasts) are commonly found on grapes and predominate at the beginning of the wine fermentation. They are, however, sensitive to alcohol and are replaced by *S. cerevisiæ* var. *ellipsoideus* as the fermentation proceeds.

The Asporogenous Yeasts

The non-sporing yeasts are commonly referred to as *Torulæ*. The generic name *Torula* was first used for a yeast-like organism by Turpin in 1838 and was soon adopted by workers in the fermentation industries and by bacteriologists. The name was, however, in earlier use by mycologists in quite a different sense, having been applied by Persoon in 1801 to a genus of dark-coloured moulds belonging to the Hyphomycetales (see Chapter VII), and, in spite of the laws of priority, the confusion has persisted. Berlese in 1895 proposed that *Torulopsis* should be substituted for *Torula* as the name of a genus of non-sporing yeasts, and this suggestion, having been adopted by a number of modern authors, will in time, no doubt, gain general acceptance.

Will (1916) first proposed a reasonable classification of these organisms and raised the group to the rank of family, the Torulaceæ, a suggestion which has been followed by a number of subsequent authors. The most recent and authoritative treatment of the non-ascosporic yeasts is by Lodder (1934), who recognizes two families and a provisional third family containing a single doubtful genus.

Carotinoid pigment produced . .	Family 1	RHODOTORULACEÆ.
No carotinoid pigment produced . .	Family 2	TORULOPSIDACEÆ.

The latter is further divided into two sub-families :

Pseudomycelium not formed .	Sub-family 1	TORULOPSIDOIDEÆ.
Pseudomycelium formed, with buds at the nodes	Sub-family 2	MYCOTORULOIDEÆ.

In Lodder's monograph the name of the first sub-family is spelt Torulopsoideæ, but has since been revised. Only the first part of the monograph has yet appeared, including the Rhodotorulaceæ and the Torulopsidoideæ but not the Mycotoruloideæ.

Rhodotorulaceæ. This family includes only one genus. *Rhodotorula.* All the species produce pigment of a carotinoid nature,

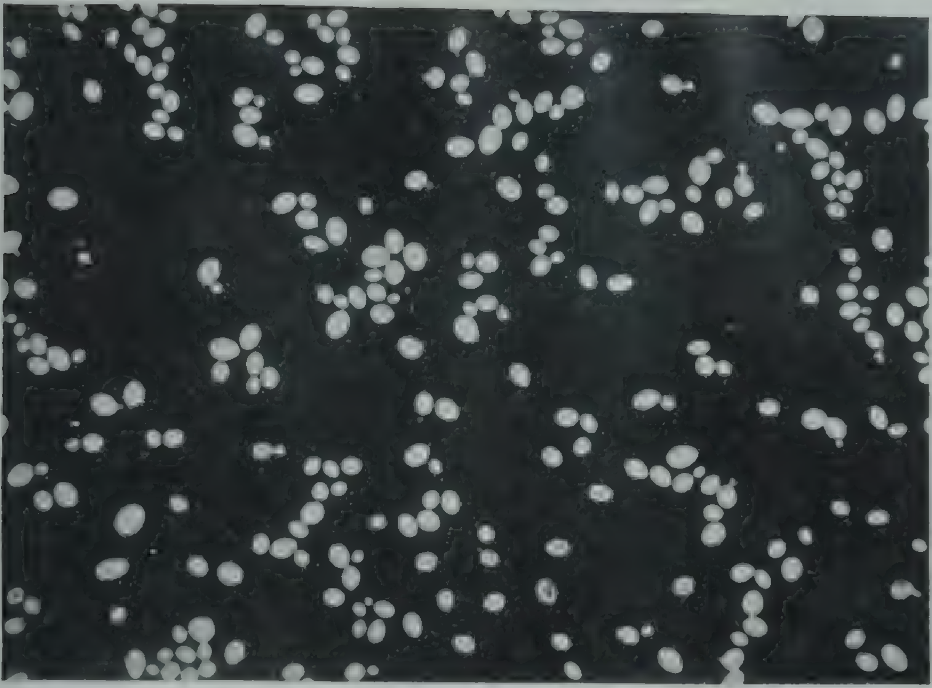

Fig. 36.—*Zygosaccharomyces sp.*—relief stained with nigrosin. × 500.

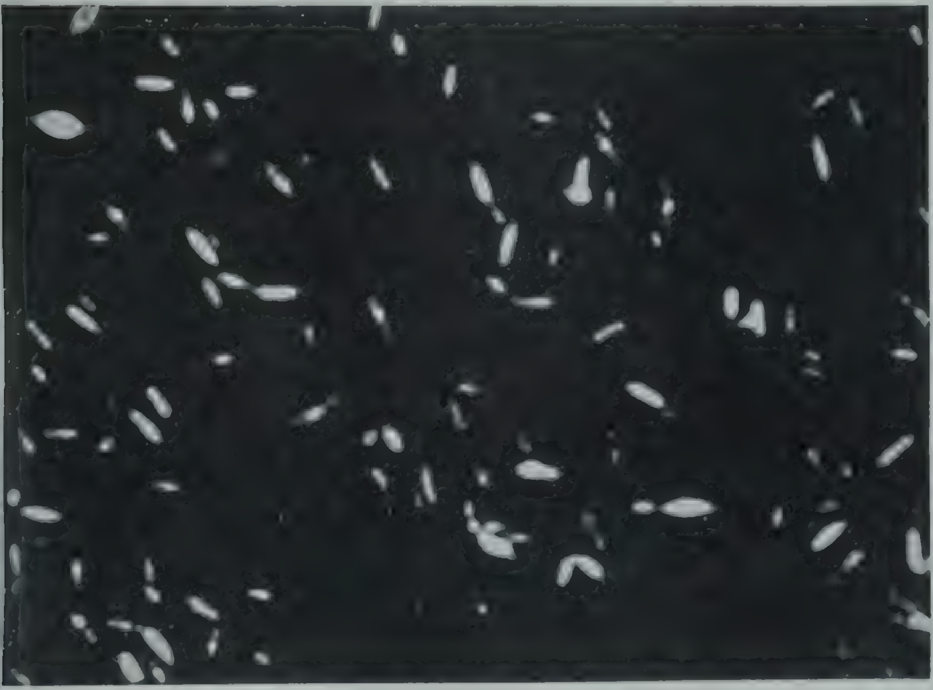

Fig. 37.—*Klœkera apiculata*—relief stained. × 1000.

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colonies being yellow, orange, pink or red. They appear to forn a homogeneous group, not closely related to the Torulopsidaceæ They are amongst the commonest of air-borne organisms and ar frequently found as contaminants of cultures of moulds and bacteria All species are non-fermentative and hence are of no practica importance, being both useless and harmless so far as is known except that they can be a nuisance in the culture laboratory. Thei chief interest is to the systematist, as they are probably imperfec forms of species of *Sporobolomyces.*

Sporobolomyces. This interesting genus was first clearly de scribed by Kluyver and van Niel (1924–5). Young colonies exactly resemble those of a typical *Rhodotorula*, but later become powdery and, if a Petri dish culture is incubated upside down, a faint mirro image of the colony is produced in the lid of the dish. This mirro colony is composed of bean-shaped spores quite unlike ordinary yeast cells. These spores are formed on small projections put ou by the oval vegetative cells and are forcibly shot off by a curiou mechanism which Buller (1933) has shown to be peculiar to the Basidiomycetes. Lodder (1934) states that strains of *Sporobolomyces,* on continued cultivation, lose the power of producing mirror colonies and become indistinguishable from *Rhodotorulæ*, whilst some typical *Rhodotorulæ* may be induced to form mirror colonies. It seems probable that the whole group of pink yeast-like organisms, including both *Sporobolomyces* and *Rhodotorula*, are primitive Basidiomycetes, some of which have lost the power of producing basidiospores, or which produce spores only under special conditions. Derx (1930) has described a second genus, *Bullera*, the species of which produce mirror colonies but lack the typical pink colour of *Sporobolomyces.* Derx states that species of both genera are of very common occurrence on straw and on leaves of various plants, but their significance in nature has not been determined.

Torulopsidoideæ. Lodder describes 7 genera belonging to this sub-family, *Klœkera*, *Torulopsis*, *Mycoderma*, *Trigonopsis*, *Asporo myces*, *Pityrosporum* and *Schizoblastosporion*, with a total of 4 species and 3 varieties. Only the first three of the genera are c sufficient importance to warrant further mention.

Klœkera. The cells are ovate to lemon-shaped, multiplying b bipolar budding (Fig. 37). Species of this genus occur on grap along with species of *Hanseniaspora*, and are obviously related t the latter. Since most strains of *Hanseniaspora* rapidly lose th power of forming ascospores when kept in culture, there seems to b stification for the view held by some workers that the two genera ould be united.

Torulopsis. This genus includes a large proportion of the "wild" asts which often appear in considerable numbers in rough cultures om mouldy materials. They are difficult to identify and are ually of little practical importance. One species, however, *T. utilis* lenneberg) Lodder, is easy to grow in bulk and is a useful source protein (see Chapter XIV). Cells are ovate, fairly large, 5–12 × 5–5·5μ; glucose and saccharose are fermented and nitrates utilized.

Mycoderma. These yeasts are probably non-sporing forms of ecies of *Pichia* and *Hansenula.* Like the latter they form dry, inkled pellicles on the surface of liquid cultures and are oxidave rather than fermentative. Cells are ovate to more or less lindrical, forming mycelium-like associations.

Mycotoruloideæ. One genus belonging to this sub-family, *ettanomyces*, has been erected to include a number of yeasts which ve been isolated from certain English and Belgian beers and ales, d which are stated to be responsible for the "after-fermentation," uilliermond 1920, Henrici 1941). The after-fermentation is a condary fermentation occurring in the bottles, in which the residual ygen is utilized and an appreciable amount of CO_2 is produced. cording to Hind (1940, p. 895) the development of the characistic flavour of fully matured stock beers is preceded by a stage which an objectionable odour is produced. Species of this group of fairly common occurrence in breweries and may constitute a irce of trouble in beers which are not subjected to a prolonged ituring process. The yeasts concerned differ markedly from *Sacc. evisiæ* which is responsible for the main fermentation. The cells of various shapes, ovoid to almost globose or elongated and nted at both ends (so-called "ogive" cells). Under anaerobic iditions fermentation is very slow, taking several months to reach npletion, but alcohol tolerance is high so that eventually a greater centage of alcohol is obtained than with *S. cerevisiæ.* Under obic conditions they oxidize alcohol to acetic acid, eventually ducing sufficient acid to kill the cultures unless the medium itains a considerable amount of calcium carbonate.

METHODS OF IDENTIFICATION

Yeasts, unlike moulds, cannot be identified from morphological a alone. Biochemical tests are necessary and, indeed, are the basis of the published keys to many of the genera. In the descri tions of methods given below, a number of points of technique, an some of the culture media, are identical with those used in identifyin moulds. These are fully dealt with in Chapter X and are no elaborated here. On the other hand, many of the methods are use for yeasts alone and are described here in preference to includin them with the more general methods.

The morphological data required are: appearance of cultures o wort agar and in liquid wort; shape and size of vegetative cell method of propagation; mode of production of asci and size an shape of ascospores. Biochemical data include: ability to fermen various sugars; in some cases ability to utilize different carbo hydrates for growth; ability to utilize nitrates as source of nitroge and alcohol as source of carbon.

Appearance of Cultures. Single colonies are grown in Pet dishes on wort agar and on wort gelatine, and details of colour an texture are recorded over a period of about 2 months. Cultures a made in liquid wort, and development of sediment, superficial rin or complete pellicle is noted.

Characteristics of Cells. Slides are made from cultures i liquid wort after 24 hours' and 3 days' incubation. The shape an dimensions of the cells are noted, also the method of propagatio whether by bipolar budding, multipolar budding or binary fissio Any tendency to form mycelium, or chains of elongated cells resen bling mycelium (pseudo-mycelium) is recorded.

Production of Ascospores. Some species, especially whe newly isolated from a natural source, form spores readily. Oth species, and most strains which have been kept for some time i artificial culture, may require special conditions, determinable onl after many trials. Very many methods have been suggested f induction of spore formation, of which the following are a selectio of the most reliable. In all the methods which depend on the use special media, transfer is made from a vigorously growing cultu on wort. All these media are starvation media, in the sense th their content of readily available source of energy is small.

1. The classical method is to transfer to small sterile blocks plaster of Paris moistened with water, very dilute wort a solution containing 2% mannitol and 0·5% K_2HPO_4.
2. Cultures are sown on sterile slices of carrot or potato, or sterilized raisins.

3. Transfer is made to Gorodkowa's agar (Glucose, 0·25%; meat extract, 1·0%; NaCl, 0·5%; agar, 2%) or to plain agar without any added nutrients, or to raisin agar.
4. Cultures on Gorodkowa's agar are irradiated with ultraviolet light, exposing different cultures for periods of 5, 10 and 15 minutes.
5. Stantial (1928, 1935) found that by continuous shaking of a suspension of washed cells of *Sacc. cerevisiæ* in dilute grape-fruit juice, a high proportion of the cells became converted to asci. Very dilute solutions of a number of sugars gave even better results than grape-fruit juice, the best being mannose and maltose. Addition of potassium acetate was favourable, especially with glucose. It was found that the concentration of the sugar solution was immaterial, provided it was low, but that the ratio of number of cells to weight of sugar must be within certain limits, 20 million cells to 1–10 mg. sugar. If acetate is added the weight must be between 5 and 25 mg. The volume with these quantities was usually about 50 ml. Although Stantial worked only with the one species, it is probable that the method would be successful with many others.

When a satisfactory procedure has been found, it is necessary to make microscopic preparations at frequent intervals from cultures which are actively sporing, in order to determine whether the process of spore formation is preceded by conjugation of cells or is parthenogenetic.

Fermentation Tests. These are usually carried out in special ubes sold for the purpose, the best known being Einhorn tubes llustrated in any catalogue of laboratory apparatus). Quite satis-actory tubes may, however, be made at little cost by anyone with little skill in glass-blowing. A quantity of yeast extract is made y autoclaving 200 g. of pressed yeast with 1 litre of tap water, ltering twice, hot and cold, and making up to 1 litre. Two per ent. solutions of the sugars to be tested are made up in this and erilized. Each fermentation tube, previously sterilized, is charged ith solution so that the closed limb is quite full and the bend is ealed so that no air can enter. A small quantity of the yeast, aken with a needle from a culture on wort agar, is introduced hrough the open short limb and the tube is incubated at 25°C. f much gas is produced this is definite evidence of fermentation, but, if only a small bubble collects, it should be tested with KOH to find whether it is CO_2 or air liberated from solution in the liquid medium.

Other Tests. Two special media, one containing nitrate as sole source of nitrogen, the other containing alcohol as sole source of carbon, are used to test the availability of these substances for growth. The first medium contains: glucose, 2%; KNO_3, 0·1%; KH_2PO_4, 0·1%; $MgSO_4.7H_2O$, 0·05%; washed agar, 2%; in distilled water. The second contains: alcohol, 3%; $(NH_4)_2SO_4$, 0·1%; KH_2PO_4, 0·1%; $MgSO_4.7H_2O$, 0·01%; in tap water.

For identification of species of the Torulopsidaceæ ability to utilize various sugars and various sources of nitrogen is tested. The simplest and most economical procedure is the auxanographic method of Beijerinck. The medium for testing sugars contains: $(NH_4)_2SO_4$, 0·5%; KH_2PO_4, 0·1%; $MgSO_4.7H_2O$, 0·05%; washed agar, 2%. About 2 ml. of a thick suspension of cells in sterile water is put in a Petri dish; 10 ml. of the medium, previously melted and cooled to 40° C., is poured in and thoroughly mixed with the suspension; when the agar has completely set very small amounts of the finely powdered sugars are placed at different spots on the surface, five or six sugars in one dish; finally the dish is incubated for a few days at 25° C. The sugars diffuse into the agar and, if utilized, stimulate into growth the cells lying within the diffusion zone. For testing nitrogen compounds, the method is the same except that the medium contains 2% glucose in place of the ammonium salt.

Microscopical Methods. Cells from cultures in liquid media may be mounted in a drop of the culture fluid. The culture is shaken to give an even suspension, a small drop is placed on a slide and a cover-glass well pressed down. Specimens from agar cultures may be mounted in lactophenol, but mounting in plain water gives better contrast. A useful method of demonstrating type of budding, and size and shape of cells, is to mount in a 5% aqueous solution of nigrosin. The cells are unstained and show as white on a blue-black ground (Figs. 36, 37). Such slides are not, of course, permanent, but remain in good condition long enough for a thorough examination. Their useful life can be considerably extended by ringing them, immediately after mounting, with shellac cement.

Examination for ascospore formation is best carried out with stained films, since vacuoles are easily mistaken for spores in unstained cells. A simple and effective method is described by Shimwell (1938). A small sample is taken with a needle from the culture nder examination and well mixed with a drop of water on a erfectly clean slide or cover-glass, then spread into a thin film and llowed to dry. The film is fixed by passing rapidly two or three imes through a bunsen flame, then flooded with 5 per cent aqueous nalachite green for 1–3 minutes, warming gently to steaming several imes. The stain is rinsed off under the tap and the film flooded with lcohol for about 30 seconds, the alcohol being rinsed off with water s soon as the vegetative cells are decolorized. The preparation may ow be dried and mounted in balsam or may first be counterstained vith 0·5 per cent aqueous safranin for a few seconds. Such a slide hows green ascospores, with the walls of the asci and the vegetative ells pink. Fig. 35 is a photograph of a preparation made in this vay, taken with a contrast filter for the green.

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CHAPTER VI

FUNGI IMPERFECTI

As already stated in Chapter I, the class usually known as the Fungi Imperfecti (Deuteromycetes of some authors) includes all those species of Mycomycetes which have no perfect fruiting stage but reproduce solely by means of asexual spores or by fragmentation of vegetative structures. In addition, it is common practice to include amongst the Fungi Imperfecti a number of species which are known to be merely the conidial stages of perfect fungi, mostly of Ascomycetes. Many *Fusaria*, for example, are imperfect stages of *Nectria*, *Calonectria*, *Gibberella* and *Hypomyces: Cladosporium herbarum* is reported to be the conidial form of *Microsphærella Tulasnei*; *Aspergillus glaucus* belongs with *Eurotium*; several species of *Penicillium* produce ascospores and there are numerous other similar connections known. From time to time fresh cases are brought to light and it is extremely probable that a large number of the Fungi Imperfecti have life cycles which are incompletely known rather than incomplete. A few purely conidial forms have been proved to be haplont strains of heterothallic species—*Monilia sitophila* and related species are examples—and it may be that there are many more similar cases as yet undiscovered, awaiting some chance observations, or systematic experiment on a large scale, for their elucidation.

Strictly speaking, the discovery of the perfect stage of a fungus, or of the connection of an imperfect form with an already known perfect species, ought to result in the separate name of the conidial form being dropped. The retention of such names, and the inclusion of the species in the Fungi Imperfecti, is purely a matter of convenience. Classification is intended to facilitate identifications just as much as it is designed to exhibit natural relationships. Many species, of which the perfect stages are known, form asci only under very special conditions of culture or only when the fungi are parasitic, conidia alone being produced in ordinary laboratory cultures. Such species would be very difficult to identify if they had to be sought n a classification based on characteristics of sexual spores and fruit-bodies, and they are best left in the Fungi Imperfecti. There are even a few species, such as *Aspergillus glaucus*, which are conveniently classified along with forms of similar conidial morphology in spite of the fact that perithecia are formed readily and abundantly under almost any conditions of culture.

It should be understood that the terms "family" and "genus" have not the same significance in the Fungi Imperfecti as in the Ascomycetes and Basidiomycetes. Species with similar cultural characteristics and conidial fructifications of the same type, and which are therefore classed together, may produce perithecia of quite different types, and hence belong in different genera of Ascomycetes. *Aspergillus glaucus* (= *Eurotium herbarum*), for example, has soft, yellow perithecia, while *A. nidulans* has perithecia which are dark coloured and brittle, and has been made the type of the genus *Diplostephanus* by Langeron. Three different types of perithecia have been described for species of *Penicillium*, and, in a rational classification, these species would be placed in three different genera. Species of the genus *Botrytis* are known to be the imperfect stages of Ascomycetes belonging to several different genera, and the same thing obtains, as stated above, for species of *Fusarium*. In addition, it is not unusual for the species in a single genus of Ascomycetes to have conidial stages which are sufficiently varied to be classed in different genera of Fungi Imperfecti. On account of these facts many mycologists use the term "form-genus" for a group of imperfect species with similar conidial fructifications, that is for what is here termed a "genus" of the Fungi Imperfecti. Moreover, it is by no means rare for a single species to produce more than one kind of asexual spores in response to varying conditions, and the different stages of such fungi have been regarded as distinct species until such time as the true life histories have been worked out. Arguing from the probability that many Fungi Imperfecti which have been incompletely studied are similarly related one to another, the term "form-species" has been used by some workers. In this and the following chapters the terms "genus" and "species" are used without qualification, but they are not to be regarded as having the same significance as when applied to perfect fungi.

The number of Fungi Imperfecti is very large, exceeding that of the Ascomycetes and, therefore, the construction of a satisfactory scheme of classification is a matter of great difficulty. The system in general use is that of Saccardo (1884). It is the only system which has been fully worked out and which includes all known genera. From several points of view it is irrational, but it is usually workable and, until someone evolves a better scheme equally complete, it is likely to retain general acceptance.

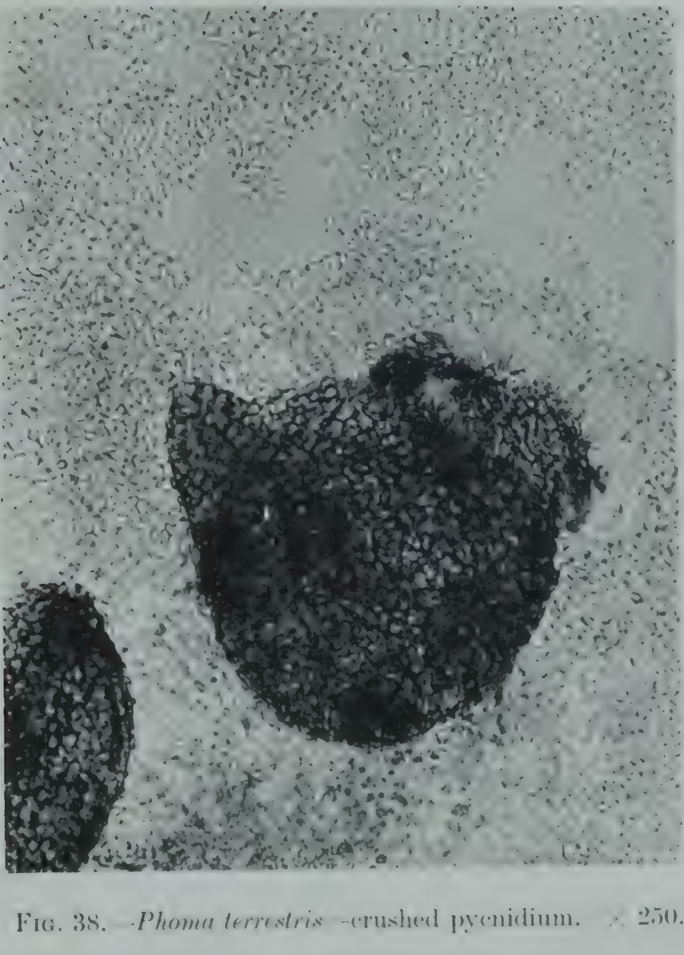

FIG. 38.—*Phoma terrestris*—crushed pycnidium. × 250.

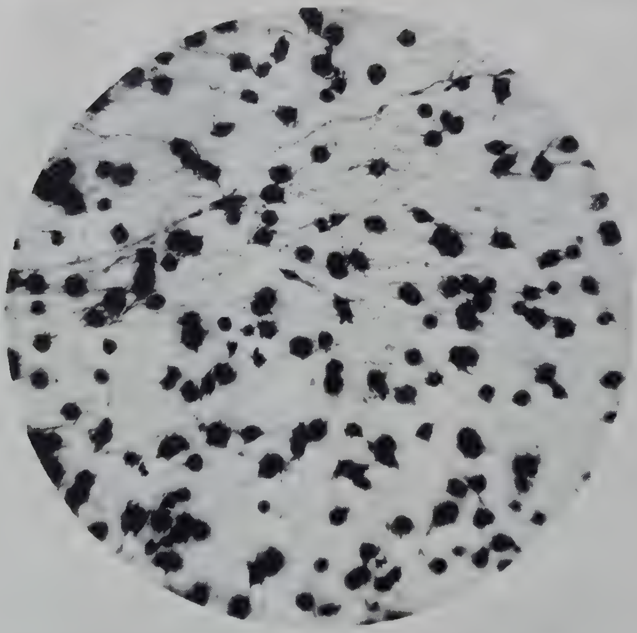

Fig. 39.—*Mycelium sterilium*—irregularly shaped sclerotia in hyphal web (Petri dish culture). × 20.

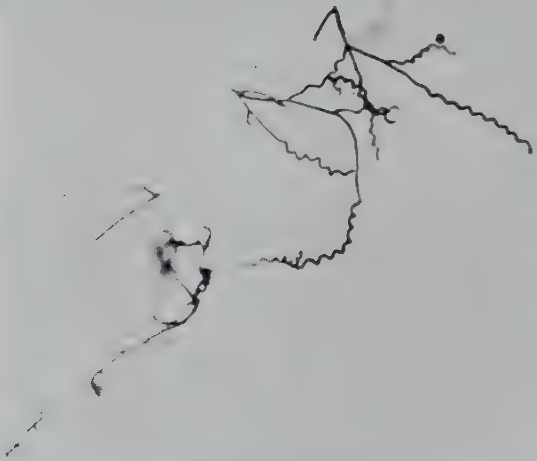

Fig. 40.—*Actinomyces sp.*—conidiophores with spiral chains of conidia (Petri dish culture). × 200.

The Fungi Imperfecti are divided into three orders, as follows: In some cases Saccardo's terminology is modernized)

onidiophores produced inside flask-shaped receptacles (pycnidia)	SPHÆROPSIDALES.
onidiophores occurring in a saucer-shaped depression in the substratum (in artificial culture these form leathery or gelatinous, fertile hyphal mats)	MELANCONIALES.
onidiophores free, arising irregularly from the mycelium	HYPHOMYCETALES.

he three orders correspond roughly with the three main divisions f Ascomycetes, Sphæropsidales with Pyrenomycetes, Melanconiales ith Discomycetes and Hyphomycetales with Plectomycetes. pecies belonging to the Melanconiales are mostly plant parasites nd are seldom encountered in industrial work. Occasionally they ppear in the laboratory in mixed cultures from infected material, ut only as isolated colonies having no practical significance. In ieir natural habitat they form a conidial layer, consisting of a mass f short conidiophores, arising from a web of hyphæ occurring in shallow depression in the surface of the host. The fructification omewhat resembles a pycnidium which has been slit and opened ut flat. When grown on artificial media such species produce ushion-shaped masses of short conidiophores, usually dark coloured, hich fall to pieces when handled. Actually there is no real criterion y which the Melanconiales may be distinguished from the Tuber-ulariaceæ (see below).

The Sphæropsidales also include many plant parasites and a umber of saprophytes found on decaying plant material, but some ecies of this order are found fairly frequently on industrial pro-ucts and a few are of practical importance. Almost all the forms countered belong to the one genus, *Phoma*. All the published ys to this order distinguish three related genera, *Phoma*, with ores less than 15μ long, parasitic on stems of plants, *Phyllosticta*, stinguished from *Phoma* only by its occurrence on leaves, and *acrophoma*, with spores over 15μ long, but the distinction between e first two has been shown to be invalid and the separation of *acrophoma* is purely artificial.

Phoma has dark-coloured pycnidia, produced singly and not on stroma, with ovate to elongate, hyaline, 1-celled spores, which e exuded as an irregular mass when a pycnidium is gently crushed der a cover-glass (Fig. 38). Identification of species is difficult at e best and usually impossible, since well over 1,000 so-called species have been described, mostly quite inadequately and seldom from pure cultures. One species, *P. pigmentivora* Massee (more correctly named *Aposphœria violacea* Bertel) is of industrial importance since it attacks freshly applied white lead paint, producing unsightly pink to purple spots, often several centimetres in diameter. Pycnidia are separate or in clusters, almost globose with scarcely any neck, 125–150μ in diameter; spores ellipsoid, 4–6 × 2–2·5μ.

The great majority of the common moulds belong to the Hyphomycetales. Saccardo divides these into four families as follows:

1. Conidiophores detached, not compacted.	
(*a*) Hyphæ colourless or in pale or bright colours	MUCEDINACEÆ.
(*b*) Mycelium, spores or both dark brown to black	DEMATIACEÆ.
2. Conidiophores compacted in fascicles.	
(*a*) Conidiophores short, forming cushion-shaped aggregates (sporodochia), often waxy to gelatinous	TUBERCULARIACEÆ.
(*b*) Conidiophores long, forming coremia .	STILBACEÆ.

Each family is sub-divided into a number of sections according to the various types of spores.

Spores pale or brightly coloured	
Spores 1-celled, globose, ovate or fusiform .	*Hyalosporæ.*
Spores 2-celled	*Hyalodidymæ.*
Spores with 2 or more cross septa	*Hyalophragmiæ.*
Spores muriform (i.e. with both longitudinal and cross septa)	*Hyalodictyosporæ.*
Spores spirally curved, septate . . .	*Hyalohelicosporæ.*
Spores forked or star-shaped	*Hyalostaurosporæ.*

Spores dark coloured

The classification is like that of the first group, the various names of sections having the prefix phæo- instead of hyalo-.

Each of the spore sections is then split into two groups.

1. Conidiophores short or obsolete, hardly distinguishable from the mycelium . . .	*Micronemeæ.*
2. Conidiophores elongate and distinct . .	*Macronemeæ.*

Within these groups the various sub-families and genera are classified according to the arrangement of spores on the conidiophores, whether solitary or in heads, produced directly on the conidiophores or on specialized branches, arranged in clusters or in chains and so on.

In addition to the multitude of species falling within the above scheme there are a number of fungi which are, so far as is known, permanently sterile. They produce mycelium and, in many cases, sclerotia, but no reproductive organs or conidia. These have no place in any of the families or groups founded on spore characteristics and are usually classed apart as *Mycelia sterilia.* Fig 39 is a photograph of such a species which has been fairly frequently met with. It forms a web of greyish hyphæ, rapidly covering the surface of the medium, with numerous sclerotia, black, irregularly placed and of various shapes. Purely mycelial forms occur fairly frequently in miscellaneous cultures, but it should not be hastily assumed that these belong with the *Mycelia sterilia.* Many of them are parasitic fungi, Ascomycetes and Basidiomycetes, which do not form spores on the usual artificial media. In addition, many of the *Fusaria* form nothing but mycelium when first isolated and can be recognized only from experience or after cultivation under conditions which will induce normal production of spores.

Saccardo's system is, of course, a key pure and simple and not n any sense an attempt at a rational classification. The artificial nature of the criteria separates, in many cases, genera which are obviously closely related, but this of itself is not a serious matter, ince the sole purpose of the key is to faciliate identifications. A graver fault is that some of the characteristics used as criteria are not so clear cut, in the majority of cases, as such a scheme demands, with the result that it is often necessary to search in more than one ection of the key before a fungus can be identified. Perhaps the most serious weakness arises from the fact that numerous genera were, of necessity, known to Saccardo only from descriptions, many f which were totally inadequate for characterization. Some of these enera have been more completely defined by modern workers, but here are still a number of common moulds which are known to resent-day mycologists merely from tradition handed on from one worker to another, and are extremely difficult to track down from eys founded on Saccardo's system.

Of the many attempts to devise a system of classification which hall be more logical than Saccardo's, only two are of sufficient mportance to be mentioned here. Vuillemin's system (1910, 1912) , based on differences in methods of spore formation instead of on he characteristics of the spores themselves. It has been worked ut most fully for a group of fungi which do not fit at all well into accardo's scheme, the pathogenic fungi, and is in general use by medical mycologists. The system has much to commend it and would probably be widely used if it could be extended to include the whole of the Fungi Imperfecti. Since the study of the pathogenic moulds is a very specialized branch of mycology, with its own extensive literature, Vuillemin's system will not be elaborated here.

Mason, in a series of publications (1933, 1937, 1941), has discussed the merits of various schemes of classification and has clarified the meanings of a number of terms used by Vuillemin and others. His main thesis, however, is that, since the conidium is obviously a means of propagation of the fungus, the mechanisms designed for dispersal of conidia are of the greatest importance for classification. The great majority of species can be broadly divided into two groups, one including all species with dry spores which are normally dispersed by wind, and the other including the species with slimy spores, normally dispersed by water. The idea has not been worked out to the extent of providing a key to all the known genera, but it has been adopted by Wakefield and Bisby (1941) for a list of Hyphomycetes recorded for Britain. The two main divisions are designated Gloiosporæ, with slimy spores, and Xerosporæ, with dry spores. Separation into Mucedinaceæ and Dematiaceæ is abandoned, but Saccardo's spore groups are retained. It is frankly recognized that in this, as in any other arrangement, there are a number of intermediate forms which defy exact classification. However, the great majority of common moulds may readily be assigned to one or other of the two groups and this scheme of classification is adopted in the key to Hyphomycetales in the next chapter.

Actinomycetes

The place of these organisms in any scheme of classification is still in doubt, but if they are to be considered as fungi then their place is in the Fungi Imperfecti. The general consensus of present opinion seems to be that they form a connecting link between the bacteria and the true fungi. They resemble bacteria in staining reactions and in their inability to grow in presence of acid. In addition, some of the pathogenic species are anærobic and thus differ sharply from the true fungi, none of which can grow in complete absence of oxygen. On the other hand, they form mycelium which shows true branching and in this respect are more closely allied to the fungi than to the bacteria.

A number of Actinomycetes are pathogenic to man and other animals, the study of these being usually regarded as the province the bacteriologist. Other species are of common and widespread ccurrence in soil and play an important part in maintaining its rtility, whilst a few are causes of plant diseases, usually known as scab." In industrial work Actinomycetes commonly appear when ant products or soil contaminated materials are plated out. In such ses they may infect cultures of moulds sown from mixed plates d are often exceedingly difficult to eradicate.

Owing to their sensitiveness to acid they do not grow on wort gar and only a few species appear on Czapek agar containing H_2PO_4, which has a *p*H of approximately 4·3. Many more of the mmon saprophytes, however, grow well on neutral Czapek agar, ade up with K_2HPO_4.

Colonies on agar media are usually slow growing and are mainly two types. One kind is somewhat shiny and moist, often wrinkled, d in appearance closely resembling colonies of certain species of *rulopsis.* Most of the parasitic forms but comparatively few of e soil organisms are of this type. The second and more common pe of colony is tough and leathery, usually with well-defined rgin, often becoming powdery on the surface as spores are formed. lonies are variously coloured, white, grey, buff, yellow, greenish, l, brown or almost black. Some species form variously coloured uble pigments which diffuse into the culture medium, but the tent to which this occurs depends a good deal on the type of bstrate. Odour is usually pronounced and characteristic, not unlike at of moist loam but often more sour and unpleasant.

The mycelium is exceedingly fine, seldom more than 1μ in diameter, d much branched, with the terminal branches segmenting to form res. In many of the common forms the spore-forming branches ome spirally coiled before segmentation occurs (Fig. 40).

Three genera are recognized by most authorities, but all the cies likely to be encountered in industrial work belong to the genus *Actinomyces.*

Detailed accounts of the Actinomycetes are given by Lieske (1921), kov (1923) and Waksman (1931).

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CHAPTER VII

HYPHOMYCETALES

s indicated in Chapter VI, the order of Hyphomycetales includes l the Fungi Imperfecti which do not form pycnidia or dark-coloured romata bearing short conidiophores, but which produce conidio-
iores irregularly on any part of the mycelium. The great majority the moulds which are of industrial importance belong here.

The total number of generic names is very large, but it is probable at many of these will sooner or later be shown to be synonyms. any genera have been founded on insufficient data, obtained from udy of single specimens in their natural habitat. In most of such ses descriptions have been hopelessly inadequate and have not ken into account the whole life histories of the fungi. The result s been that some of the commoner moulds are known by tradition d are difficult to recognize from published descriptions, whilst any genera exist only as names and are totally unrecognizable at e present day.

The simple key given here takes into account only the more mmon forms, such as are fairly often found in industrial work. or identifications of fungi which are not included, the reader should nsult one of the works of reference cited in Chapter XV.

The key is based primarily on Mason's scheme of classification e p. 66), as it is considered that this scheme is easier to use than Saccardo's, with its division of the order into four families, based characteristics which are seldom sharply defined. The distinction tween Mucedinaceæ and Dematiaceæ is often puzzling to the ginner, because some species of the former have dark spores, ilst many of the Dematiaceæ have pale or hyaline mycelium and ny others are of a colour which it is difficult to class as pale or k. However, in order to facilitate reference to the larger taxo-
nic works, the place in Saccardo's system of each genus described e is indicated immediately after the name, thus: (Muc.), (Dem.), ilb.) or (Tub.).

KEY TO THE MORE COMMON GENERA OF HYPHOMYCETALES

Spores occurring in slimy or waxy masses, in false, more or less spherical heads, or in irregular clumps . . GLOIOSPORÆ . . . 2

	Spores dry, borne singly, in chains, or in clusters	XEROSPORÆ	10
2.	Spores one-celled		3
	Spores septate, sickle-shaped, borne in irregular masses or in cushion-shaped sporodochia (1-celled microconidia often present in addition)	*Fusarium* (p. 90).	
3.	Spores formed by fragmentation of mycelium; colonies soft and yeast-like, at least when young	*Oospora* and *Oidium* (p. 72).	
	Spores distinct from the mycelium		4
4.	Spores borne on all parts of the mycelium; colonies slimy, pale when young, becoming black	*Pullularia* (p. 78).	
	Spores borne on more or less definite conidiophores		5
5.	Conidiophores unbranched, arising from all parts of trailing hyphæ; spores in balls		6
	Conidiophores branched, at least at the tip, or bearing whorls of specialized, conidium-producing cells (sterigmata)		7
6.	Spores colourless; colonies white or pale coloured	*Cephalosporium* (p. 79).	
	Spores dark coloured; colonies pale at first then dark	*Gliomastix* (p. 79).	
7.	Spores colourless or pale coloured		8
	Spores dark coloured, borne on thick sterigmata	*Stachybotrys* (p. 82).	
8.	Spores at first in chains, later forming dense masses; conidiophores several times verticillate at tip	*Gliocladium* (p. 133).	
	Spores in small balls		9
9.	Branching of conidiophores irregular; colonies bright green	*Trichoderma* (p. 79).	
	Branching more or less regularly verticillate; colonies variously coloured	*Verticillium* (p. 80).	
10.	Spores 1-celled		11
	Spores regularly 2-celled		21
	Spores with more than 2 cells		22
11.	Spores formed by fragmentation of mycelium, usually forming branched chains, dark	*Torula* (in part) (p. 72).	
	Spores distinct from the mycelium		12
12.	Spores borne singly on all parts of mycelium; colonies white or pale coloured	*Sporotrichum* (p. 78).	
	Spores in clusters or in chains		13

3.	Conidiophores compacted into definite coremia	14
	Conidiophores detached or, at most, in small inconspicuous fascicles	15
4.	Coremia with green fertile heads	*Penicillium* (in part) =*Coremium* (p. 90).
	Coremia white, fertile along whole length	*Isaria* (p. 90).
	Coremia dark, with definite stalk and head	*Stysanus* (p. 90).
5.	Spores in grape-like clusters ; colonies greyish	*Botrytis* (p. 80).
	Spores in definite chains, simple or branched	16
6.	Spores multiplying by budding, forming branched chains	17
	Spores formed successively by abscission from sterigmata, in unbranched chains	18
7.	Colonies mostly pale coloured ; spores colourless or nearly so (additional spores by fragmentation of mycelium in old cultures)	*Monilia* (p. 78).
	Colonies greyish or fuscous ; spores mostly in detached chains, not forming dense heads	*Torula* (in part) (p. 72).
	Colonies dark green or grey-green ; spores in dense tree-like heads	*Cladosporium* (p. 80).
8.	Conidiophores arising from specialized foot-cells, usually non-septate, terminating in a swelling which bears the sterigmata	*Aspergillus* (p. 93).
	Conidiophores not from foot-cells, septate, bearing terminal sterigmata	19
9.	Sterigmata in definite broom-like whorls	*Penicillium* (p. 129).
	. . and young stages of	*Gliocladium* (p. 133).
	Sterigmata sometimes in whorls, sometimes disposed irregularly ; conidiophores dèfinite or lacking ; colonies white or buff, never green	20
).	Spores large, with thickened ring and pore ; sterigmata tubular	*Scopulariopsis* (p. 133).
	Spores ovate, without ring or pore ; sterigmata ending in long beaks	*Pœcilomyces* (p. 134).
1.	Spores colourless or pale coloured in mass, borne in terminal clusters	*Trichothecium* (p. 80).

Spores dark, forming branched chains and eventually dense heads (young spores usually 1-celled) . . *Cladosporium* (p. 80).

22. Spores with cross septa only, produced singly but finally forming clusters like bunches of bananas, dark 23

Spores with both cross and longitudinal septa, dark coloured 24

23. Spores smooth . . . . *Helminthosporium* (p. 89).

Spores warted . . . . *Heterosporium* (p. 89).

24. Spores rounded, not beaked, in small clusters . . . . . *Stemphylium* (p. 90).

Spores beaked or pointed, in short, often branched, chains . . *Alternaria* (p. 89).

Oidium and Oospora (Muc.). There is some difference of opinion as to the use of the two names. Many authorities reserve the name *Oidium* for parasitic forms, mostly imperfect stages of Erysiphaceæ and known as powdery mildew, and the name *Oospora* for saprophytes. Others regard species in which the mycelial fragments are more or less rectangular as *Oidium* and species with rounded cells as *Oospora*. It is probably best to follow the former practice.

Oospora lactis (Fresenius) is well known in the dairy industry and is found on all types of milk products. It grows well on wort agar, producing a thin, spreading, somewhat slimy, creamy white colony. Very young cultures show fairly long mycelial strands, whilst older cultures consist entirely of short rectangular fragments, which take the place of spores (Fig. 41).

O. crustacea Bulliard is commonly found on cheese rind, where it forms an orange-coloured, powdery growth. On culture media it grows best at about 18°C., spreading slowly at first, then more rapidly, the colour being bright orange to scarlet. At higher temperatures many strains readily form saltants, dark brown or white, which do not revert to the orange form. The cells of the young mycelium become rounded before falling apart, and look like long chains of ovate conidia (Fig. 42).

Torula (Dem.). Two types of spore production are found in this genus as usually interpreted. In the first (the true *Torula* according to Mason 1941; type species *T. herbarum* Persoon) the first spore is formed on the end of a short hypha and further spores are produced by a process of budding, as in *Cladosporium*, forming unbranched or branched chains. The spores are almost globose and

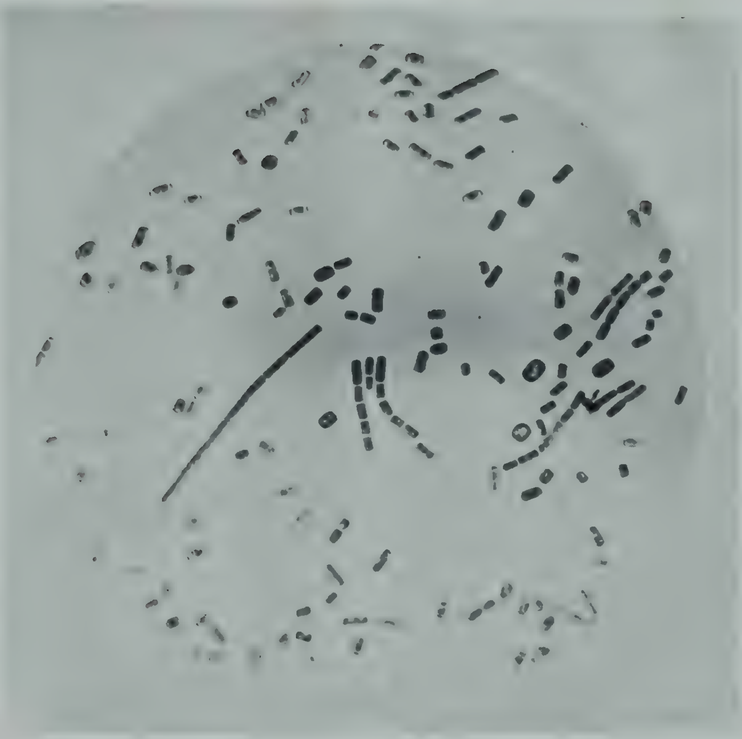

Fig. 41.—*Oospora lactis*—fragmenting mycelium and oidia. × 250.

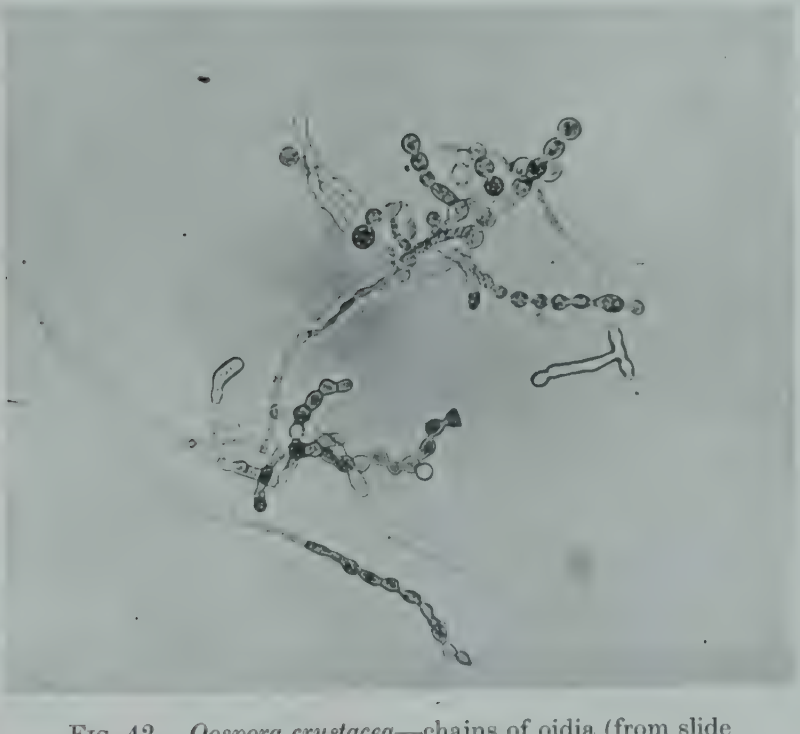

Fig. 42.—*Oospora crustacea*—chains of oidia (from slide culture). × 250.

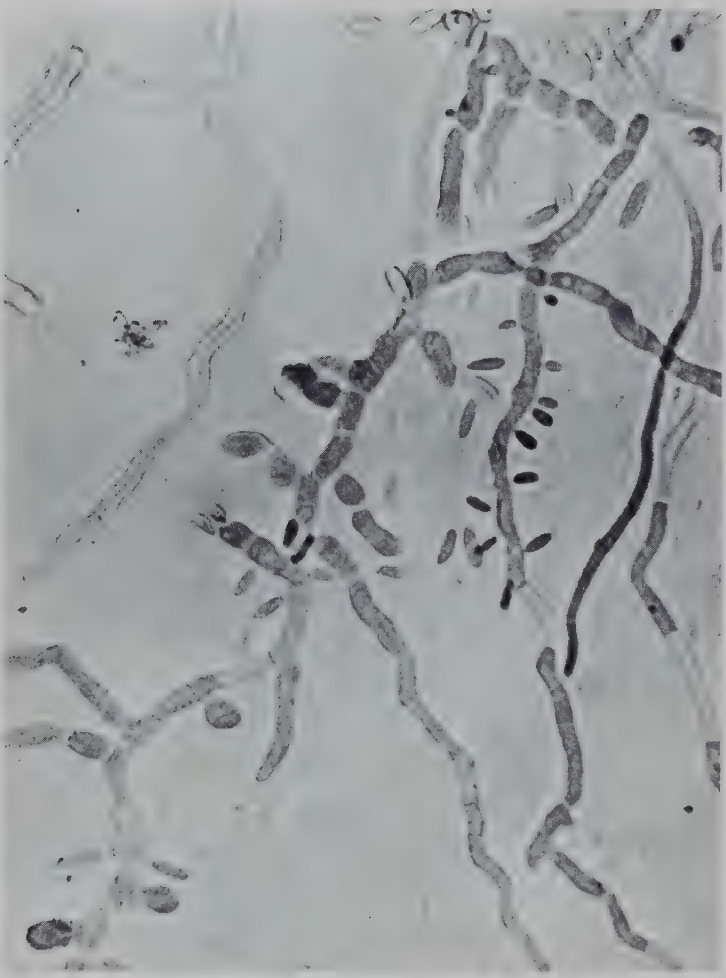

FIG. 43.—*Pullularia pullulans*—culture on "Cellophane." ×500.

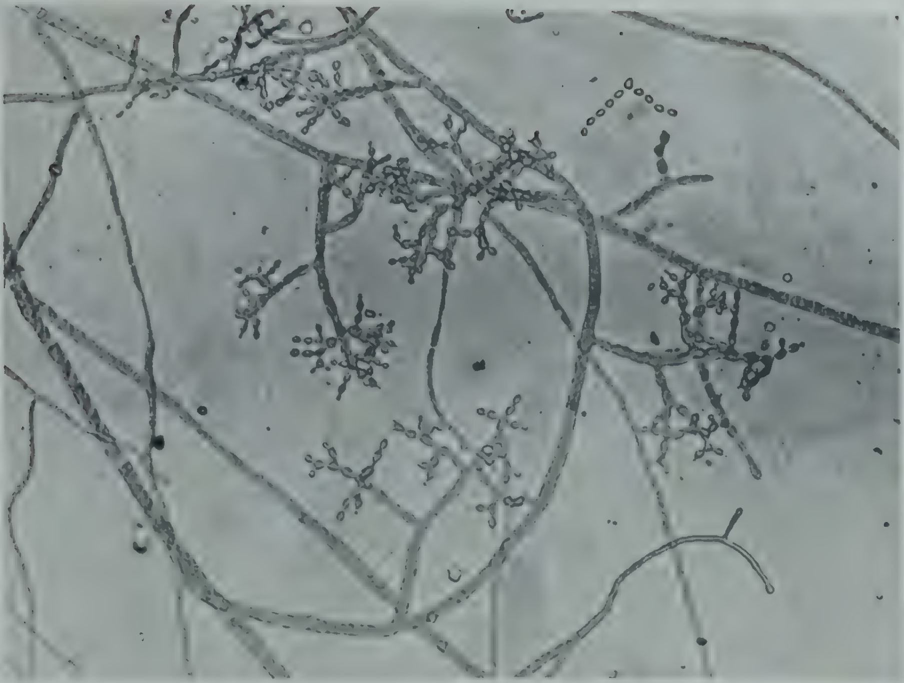

Fig. 44.—*Monilia sitophila*—young culture (on slide) showing budding of spores. × 200.

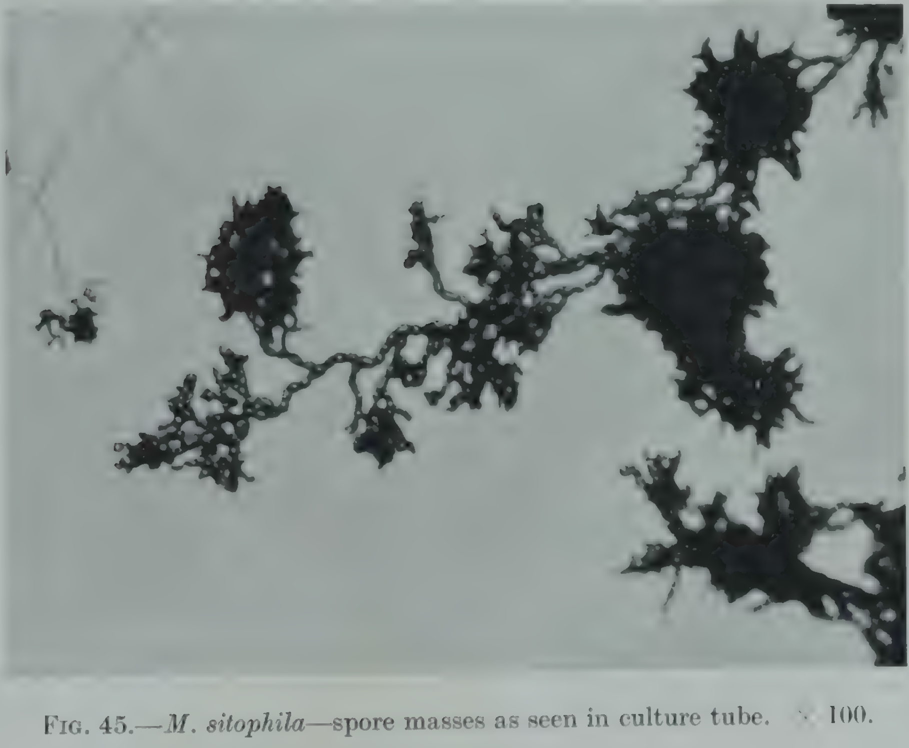

Fig. 45.—*M. sitophila*—spore masses as seen in culture tube. × 100.

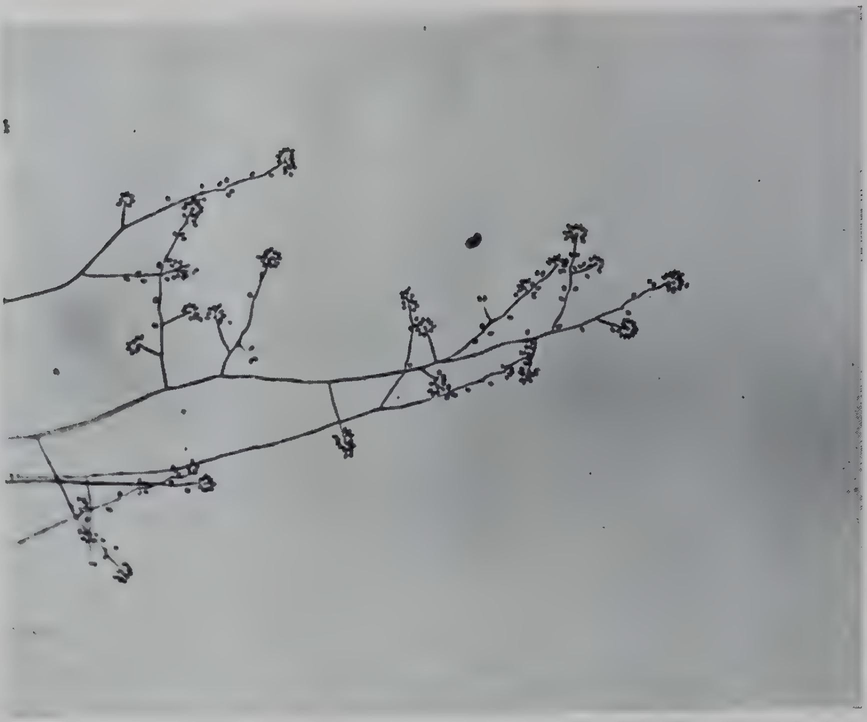

FIG. 46.—*Sporotrichum sp.*—conidia (slide culture). × 250.

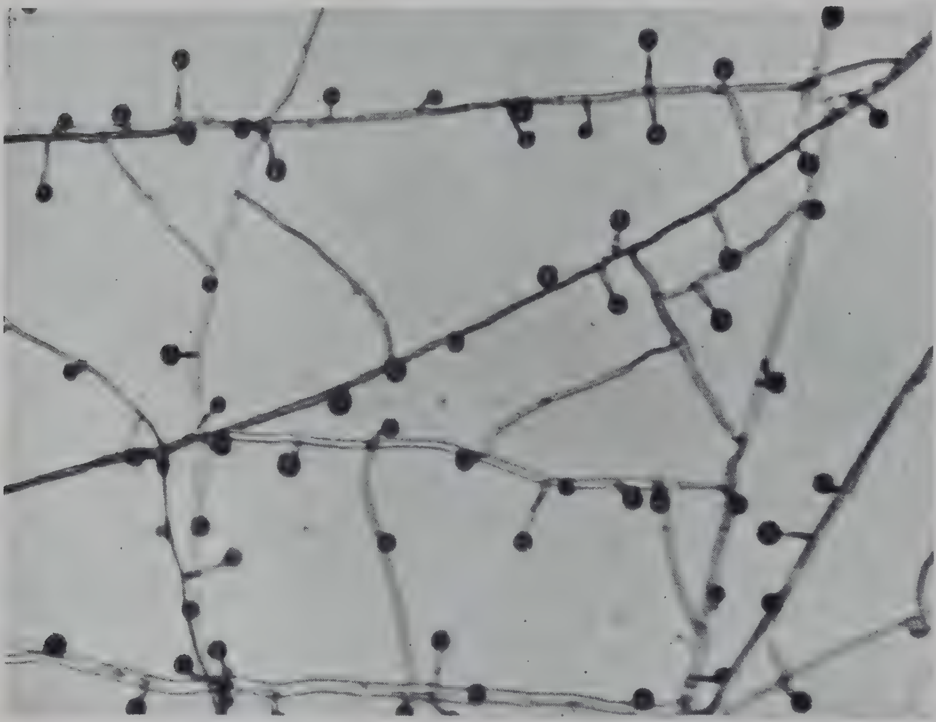

FIG. 47.—*Cephalosporium sp.*—fertile hyphæ with small spore-balls, as seen in living culture. × 250.

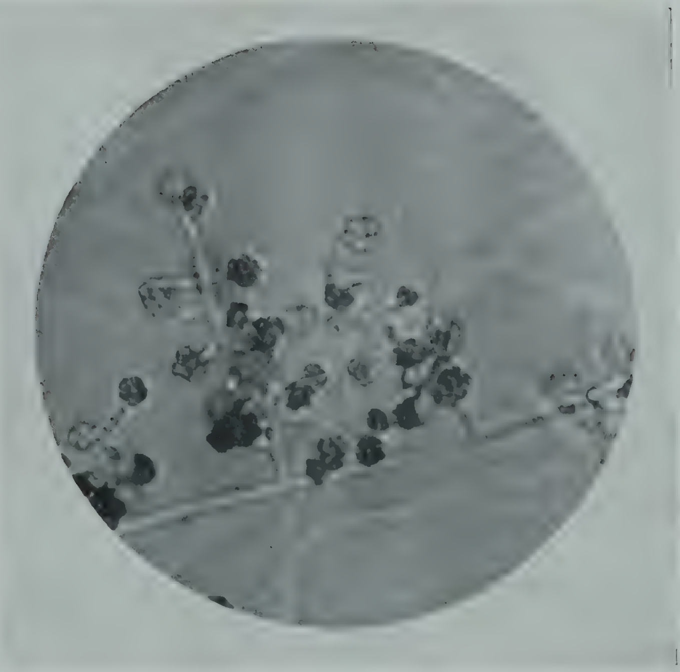

FIG. 48.—*Trichoderma viride*—conidiophore with balls of spores (slide culture). × 500.

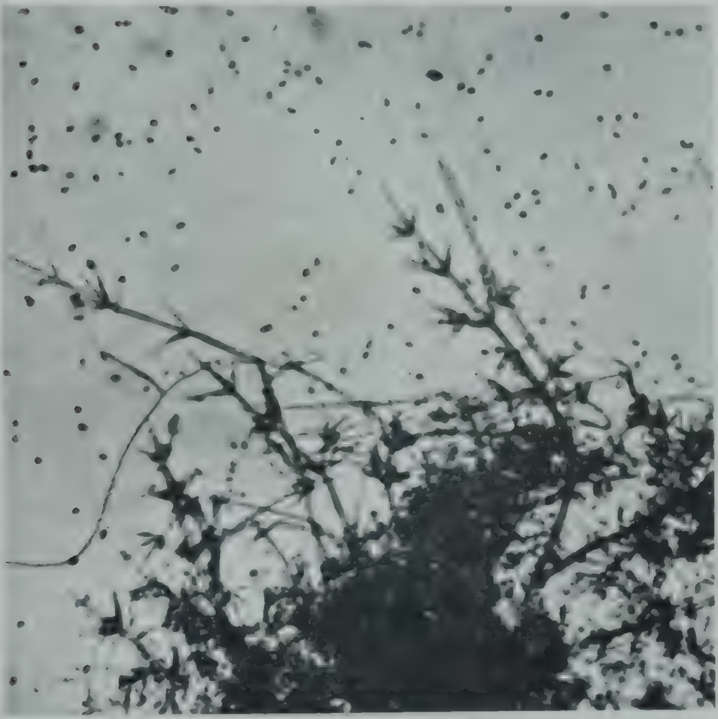

FIG. 49.—*Verticillium cinnabarinum*—conidiophore. × 250.

faintly spiny when young. Colonies are pale at first, then greyish or fuscous. In the second type aerial hyphæ become differentiated into spores from the tip backwards, the spores being ovate to barrel-shaped (as in some species of *Oospora*) and dark coloured.

Pullularia (Dem.). *P. pullulans* (De Bary and Low) Berkhout, commonly known as *Dematium pullulans*, is a fairly frequent accompaniment of other dark-coloured moulds, such as *Alternaria* and *Stemphylium*, on damp walls. Colonies are at first dirty white and mucoid, somewhat resembling yeast colonies; soon dark-green streaks appear, rapidly turning black, and finally the whole surface becomes black and more or less leathery. Young cultures consist of fine, hyaline hyphæ. The appearance of colour is due to the growth of thicker, dark-coloured hyphæ, which are twisted, much septate, often thickened at the nodes and bearing numerous ovate, hyaline conidia as lateral buds. Structure is not easy to make out, since the hyphæ break up and shed the conidia in fluid mounts (Fig. 43).

Monilia (Muc.). One species, *M. sitophila* (Montagne) Saccardo, is best known as the red bread mould. From time to time outbreaks of the infection occur in bakeries but, more often, this species attacks wrapped and, particularly, sliced bread, and is noticed only after the bread has left the bakery. In an amazingly short time the bread becomes covered with a characteristic pink, loose-textured growth. According to Shear and Dodge (1927) it also attacks sugar cane bagasse in store, forming long pink festoons from the bales. It is commonly found as an aerial contaminant and can be a great nuisance in a culture room since it spreads with extreme rapidity on most media. It forms loosely floccose masses of a pale pink to salmon-pink colour. Ovate conidia are borne at the ends of the aerial hyphæ (Fig. 44). These increase by budding and eventually form enormous, irregular masses (Fig. 45). Later the mycelium breaks up at the septa, though not usually so freely or completely as *Oospora*. Shear and Dodge (1927) have shown that *M. sitophila* is the imperfect form of an Ascomycete, *Neurospora sitophila*, but, since the latter is heterothallic, it is seldom that ascospores are found. They have also shown that there are several species of *Neurospora* with very similar conidial stages. Of these *N. tetrasperma* is homothallic, but single strains of the others cannot be recognized with certainty unless they are grown in a series of cultures along with authentic + and — strains of the various species.

Sporotrichum (Muc.). Several species are known, most of them saprophytes, but at least one species is parasitic on man, causing skin disease known as sporotrichosis. The saprophytic forms grow s cream-coloured colonies, at first wet and yeast-like, later becoming ry and more or less powdery. Spores, mostly small and pear-haped, are produced singly or in small clusters from all parts of he mycelium (Fig. 46).

Cephalosporium (Muc.). In this genus the conidiophores are hort branches from trailing hyphæ, bearing at their tips approxinately spherical masses of spores, which are usually held together y a sticky fluid (Fig. 47). The spores are produced in great numbers nd the balls fall to pieces when touched. A number of species have een described, but they are mostly ill-defined and difficult to lentify. The forms usually encountered spread very rapidly on iboratory media, and when occurring, as they often do, as coniminants of other cultures, are not easy to eradicate.

Gliomastix (Dem.). The only common species of this genus, *. convoluta* (Harz) Mason, is frequently found in soil and on soil-ontaminated materials. It was originally included in the genus *'orula* but differs from all species of the latter in the method of oore production. The mycelium is white or pale coloured, showing ark, almost black, patches as spores develop. Short sterigma-like ranches arise from trailing hyphæ or ropes of hyphæ and from the ps of these conidia are cut off successively, forming, during the rowing period, slimy balls. In old cultures the conidia tend to rm chains. The ripe spores are dark coloured, ovate, 4–5 × 3–4μ, ith the colouring matter aggregated in small granules which tend become detached in fluid mounts. The general morphology, except r colour, is that of a *Cephalosporium* (Fig. 50).

Trichoderma (Muc.). The spores are produced in slimy balls, as *Cephalosporium*, but the conidiophores are irregularly branched, e final branches being true sterigmata, abstricting the spores ccessively. Three names are frequently encountered in the erature, *T. lignorum* (Tode) Harz, *T. Koningi* Oudemans and *T. ride* Persoon, distinguished, according to various authors, by colony lour and size of spores. Bisby (1939) has shown that, when a large imber of strains are examined, there are no sharp differences to observed, and that the range of variation does not exceed that ich may be expected in a single cosmopolitan species. He considers e genus to be monotypic, and the correct name of the species to *T. viride* Pers. ex Fries.

This species is commonly found on fallen timber and is also of idespread occurrence in soil, especially very damp soils, where it can parasitize other fungi (see Chapter XI). It appears fairly frequently in the laboratory on a variety of materials, and grows well on most common media. Colonies spread rapidly, forming a somewhat thin mycelial layer with irregularly shaped patches of verdigris green, this being the colour of the ripe spores in mass. The spores are produced most freely at the shallow end of slopes and the spore masses in some strains may remain white for some time, becoming green only tardily. The fragile spherical heads contain 10 to 20 spores, spherical or slightly ovate, 2·5–3μ in diameter (Fig. 48). Chlamydospores are formed by most strains, these being terminal or intercalary and 7–14μ in diameter. On some substrates the reverse and medium are bright yellow to brown. Some, but not all strains, have a distinct odour of coco-nut.

Verticillium (Muc.). The spores are borne as in *Trichoderma*, but the conidiophores are longer and verticillately branched (Fig. 49). *V. glaucum* Bonorden forms rapidly spreading colonies of a dense cottony texture, gradually turning glaucous green in patches as the spores ripen. *V. cinnabarinum* (Corda), commonly known as *Acrostalagmus cinnabarinus*, forms thin, velvety colonies of a brick-red colour, with elliptical spores, about 3μ by 1·5μ.

Botrytis (Muc.) Only one species, *B. cinerea* Persoon, is of frequent occurrence. It is a common saprophyte and also a serious parasite of many kinds of plants. In culture it grows as floccose colonies, pale, dirty brown in colour, with fairly long, stiff conidiophores, irregularly branched at the end and bearing clusters of ovate conidia, the whole fructification resembling a bunch of grapes (Fig. 51). Typical sclerotia begin to develop after a few days as small knots of mycelium, which rapidly increase in size, turn dirty green, then black and finally may attain several millimetres in diameter.

Trichothecium (Muc.). There is one common species, *T. roseum* Link (Syn. *Cephalothecium roseum* Corda). Colonies are somewhat thin, floccose, wide spreading, white at first, then slowly pale pink. The erect conidiophores bear terminal clusters of spores directly attached to the tip (Fig. 52). The spores are 2-celled, roughly oval with a nipple-like projection at the point of attachment, 18–20μ × 8–10μ (Fig. 53).

Cladosporium (Dem.). A large number of specific names have been bestowed in this genus, but it is certain that the majority are to be regarded as synonyms. A few of the parasitic forms are fairly readily distinguishable but, until such time as someone sorts out

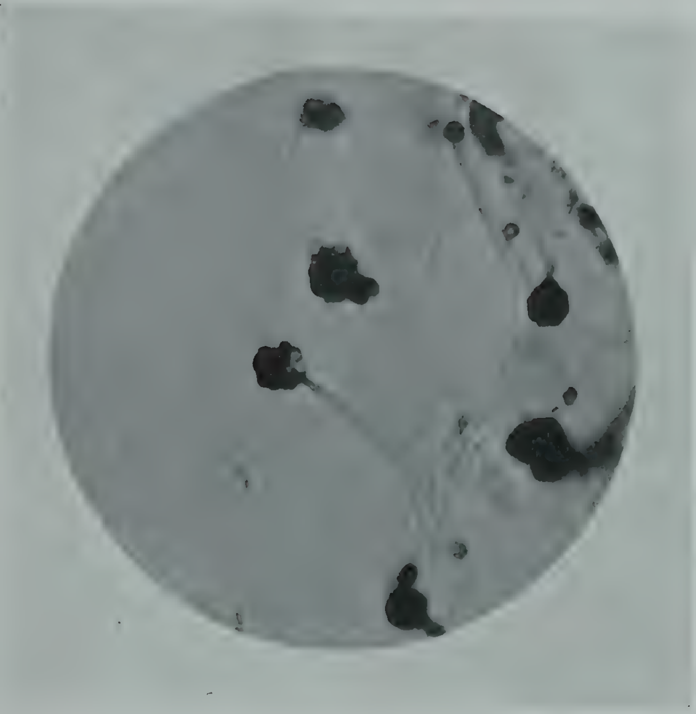

FIG. 50.—*Gliomastix convoluta*—conidiophores with spore balls. × 500.

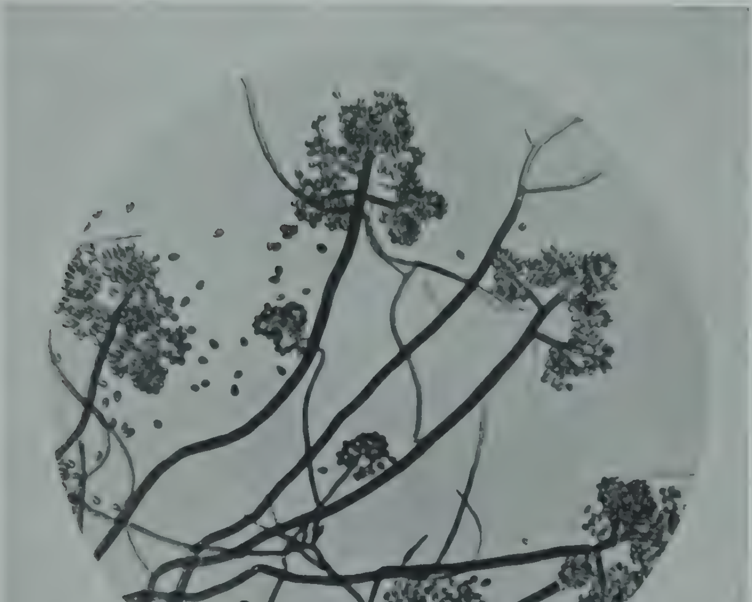

FIG. 51.—*Botrytis cinerea*—typical conidiophores. × 100.

the genus according to modern ideas, it is probably best to lum together all the common, green, saprophytic forms as *C. herbarum* In this connection Brooks and Hansford (1922) give the results of careful comparison of a number of strains and conclude that the should be all regarded as belonging to a single species. *C. herbarum* Link is a common organism of very widespread occurrence, bein found on textiles, rubber, leather, numerous foodstuffs and all kind of decaying materials of vegetable origin. It grows over a wid range of temperature, and has been reported as infecting mea stored at −6°C. On culture media the usual type of colony i somewhat restricted in growth, thick velvety, in colour varyin from deep rich green to dark grey green, with reverse a curiou opalescent blue black or greenish black. When examined dry unde the microscope, the spores are seen to occur in large, almost tree-lik clusters (Fig. 54). When mounted, the structure breaks up completely, the spores becoming detached and the hyphæ breaking int short rod-like fragments. All parts of the fungus are dark brownis green to dark brown. Examination of very young cultures will show that the ovate spores increase by budding (Fig. 55) in a manne reminiscent of the yeasts, eventually forming much-branched chains The young spores are mostly non-septate, the older ones frequentl 2-celled. Originally the name *Cladosporium* was restricted to strains with septate spores, *Hormodendron* being the name given to forms with one-celled spores, but it is now known that both types o spore occur regularly in a single culture and, therefore, the name *Hormodendron* becomes a synonym.

Stachybotrys (Dem.). The vegetative hyphæ and conidiophores are hyaline or nearly so. The short conidiophores bear at thei tips whorls of thick sterigmata, hyaline or partially brown, each bearing a spore or a short chain of spores. Several specific names have been in common use but Bisby (1943) maintains that all th forms with black or brown spores belong to one species, *S. atr* Corda *ex* Fries. The spores are ellipsoidal to sub-globose, ofte slightly curved, dark brown to black, smooth when young, mostl rough in old cultures, 8–12 × 5–7μ. (Fig, 56). This species is fairl common on paper and other cellulosic materials. It has been foun frequently on rotting sand-bags and has been shown to decompo cellulose very rapidly in presence of other organic food material.

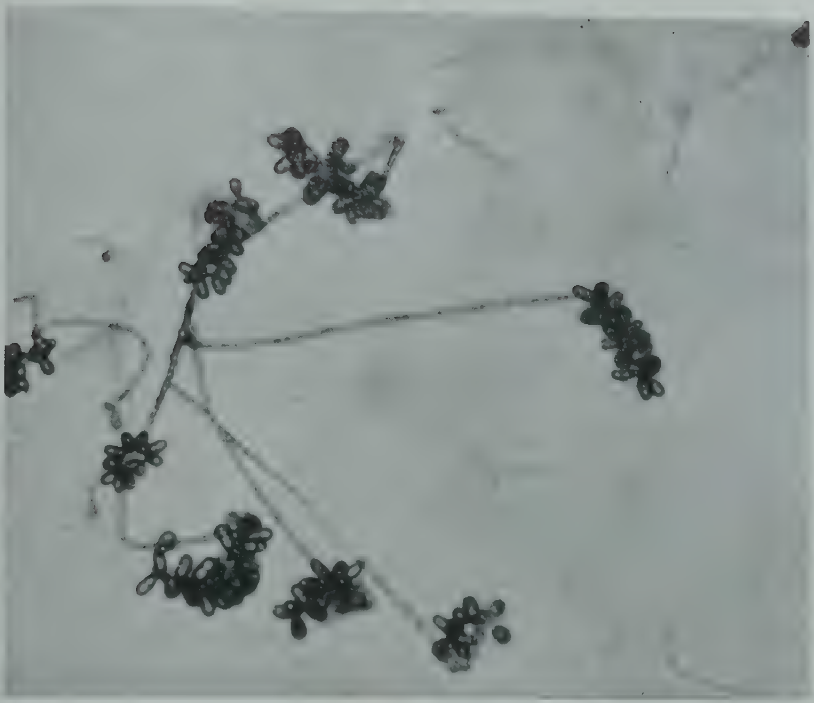

FIG. 52.—*Trichothecium roseum*—conidiophore as seen in dish culture. × 200.

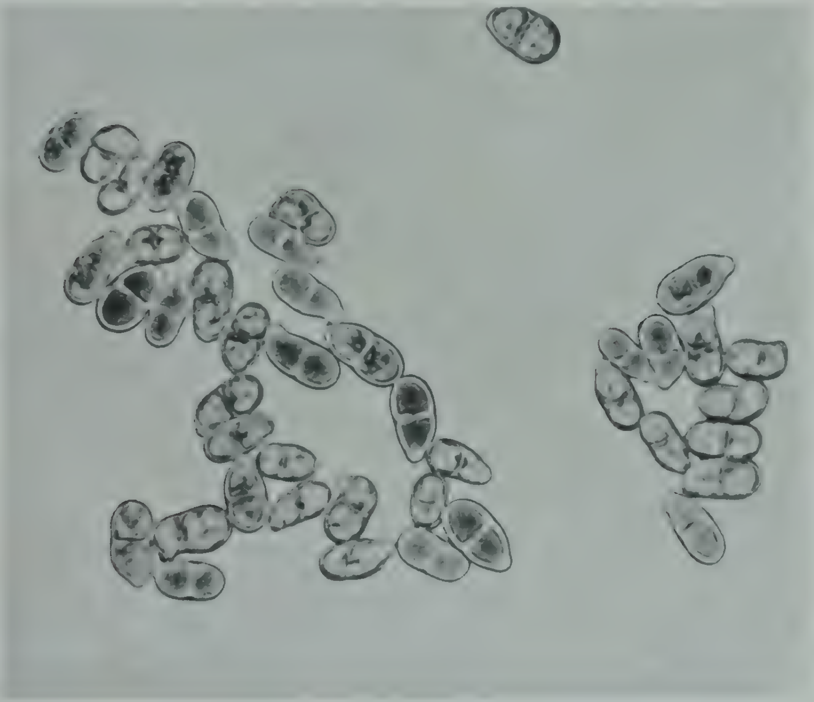

FIG. 53.—*T. roseum*—spores. × 500.

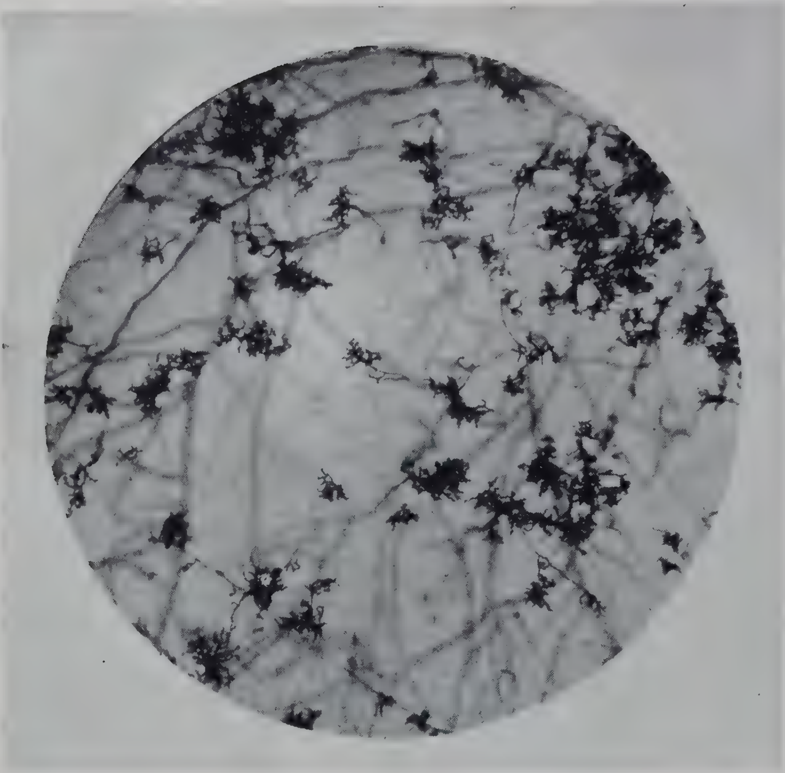

Fig. 54.—*Cladosporium herbarum*—spore heads as seen in dish culture. × 50.

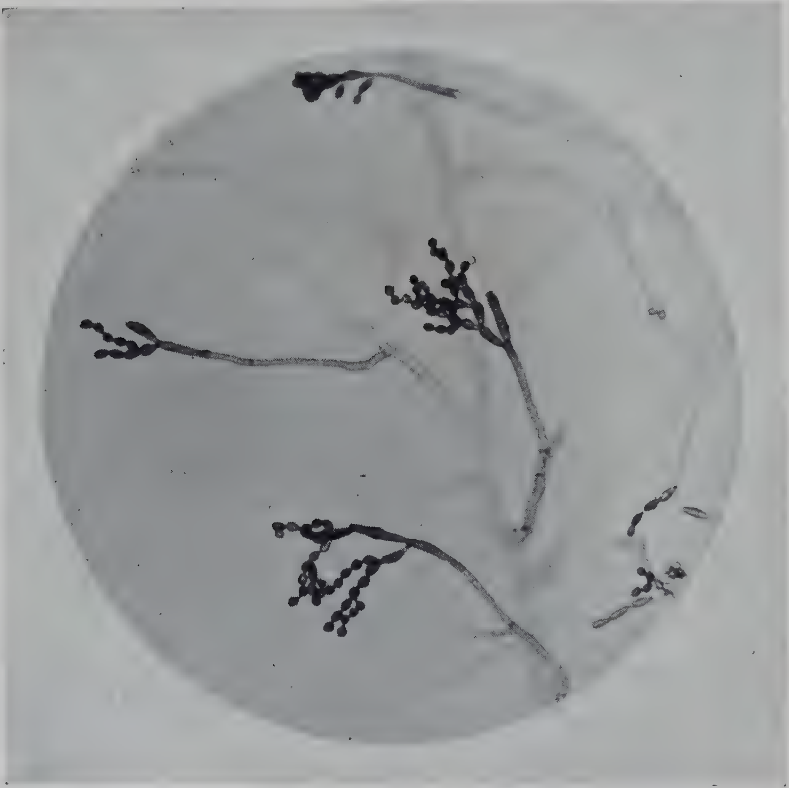

Fig. 55.—*C. herbarum*—young conidiophores showing budding of spores (slide culture). × 250.

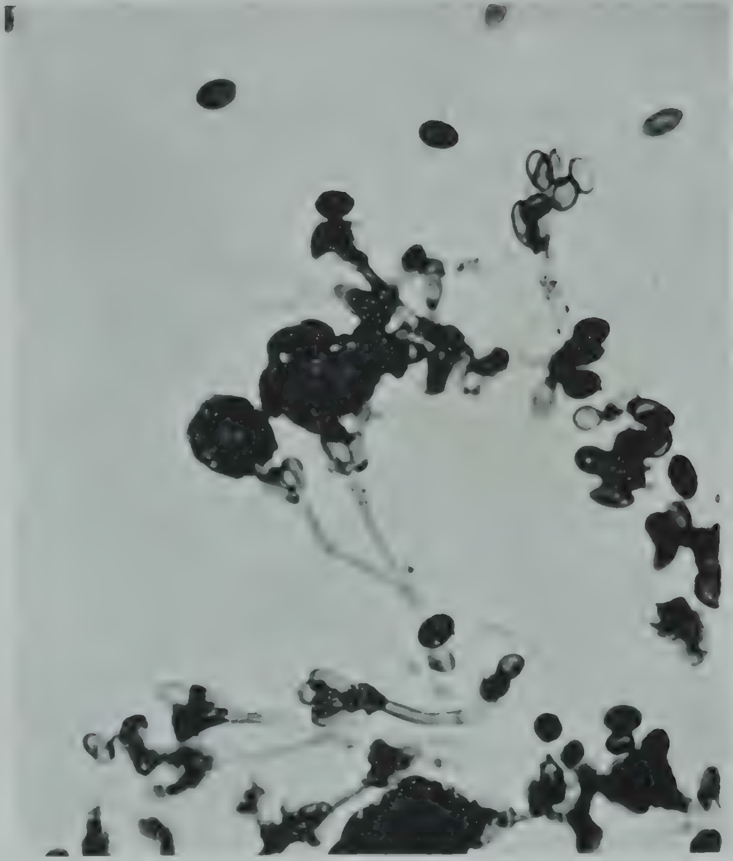

FIG. 56.—*Stachybotrys atra*—conidiophores, showing sterigmata and dense masses of dark spores. × 500.

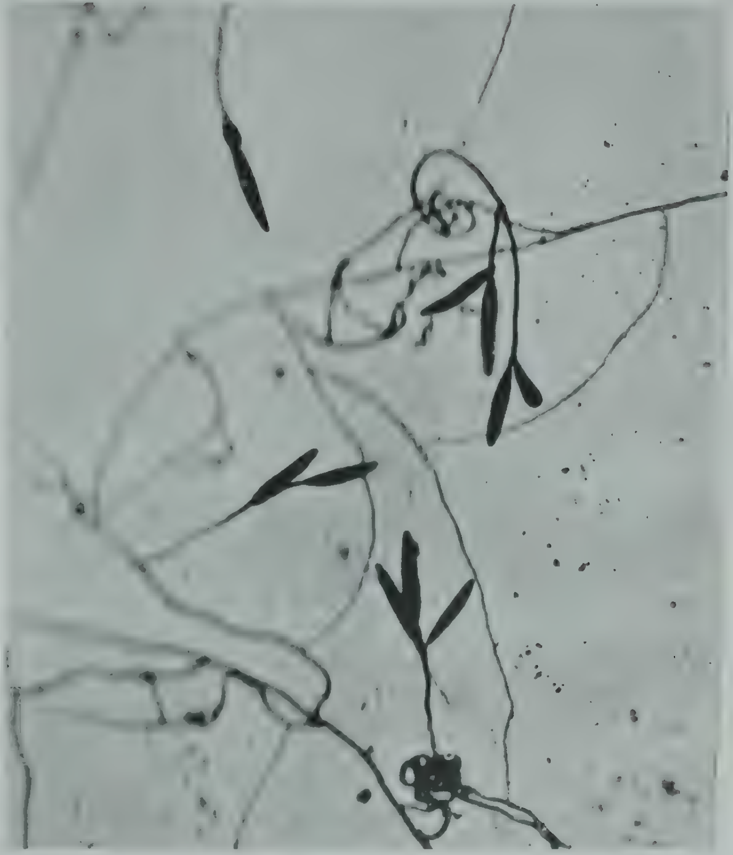

FIG. 57.—*Helminthosporium monoceras*—young conidiophores, showing "successive" formation of spores (from tube culture). × 90.

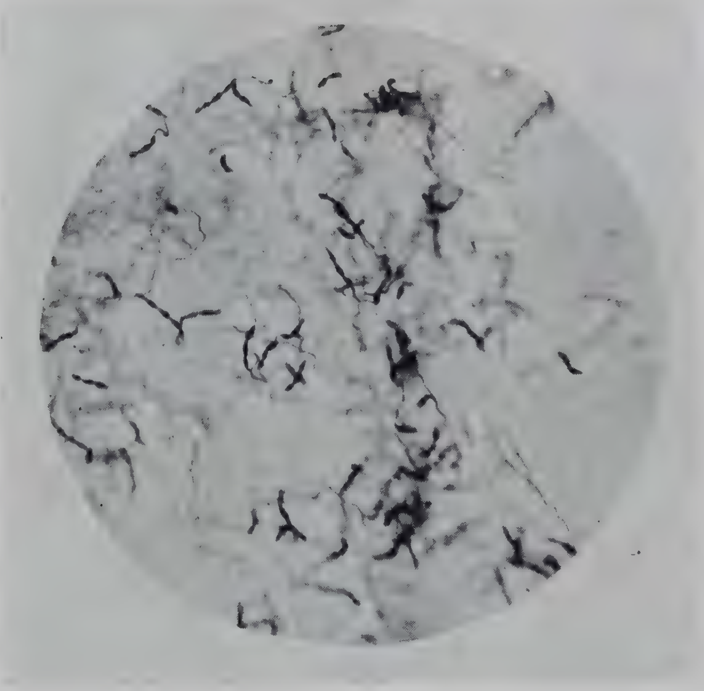

FIG. 58.—*Alternaria brassicæ*—spore chains as seen in dish culture. × 42.

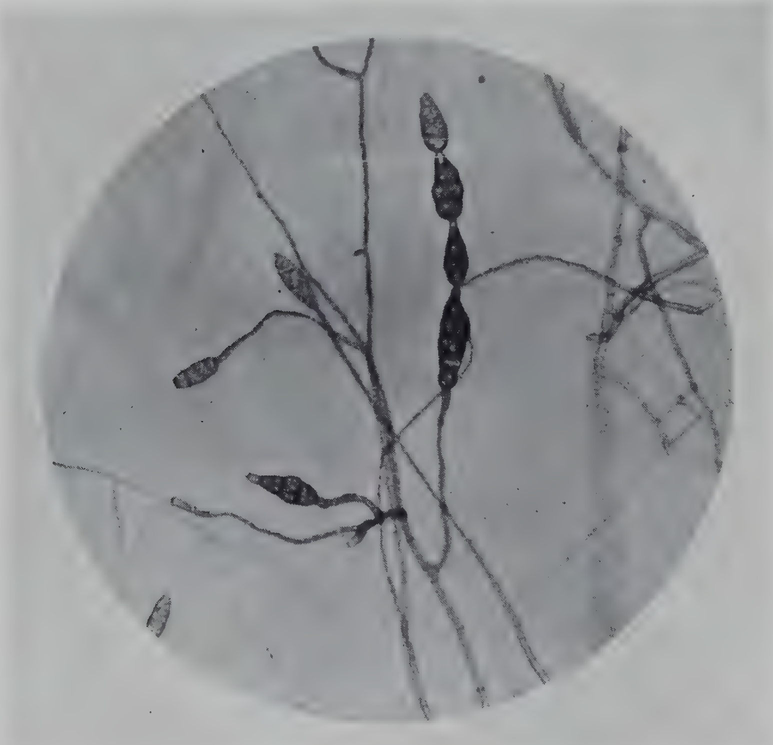

FIG. 59.—*A. brassicæ*—typical short chain of spores (slide culture). × 250.

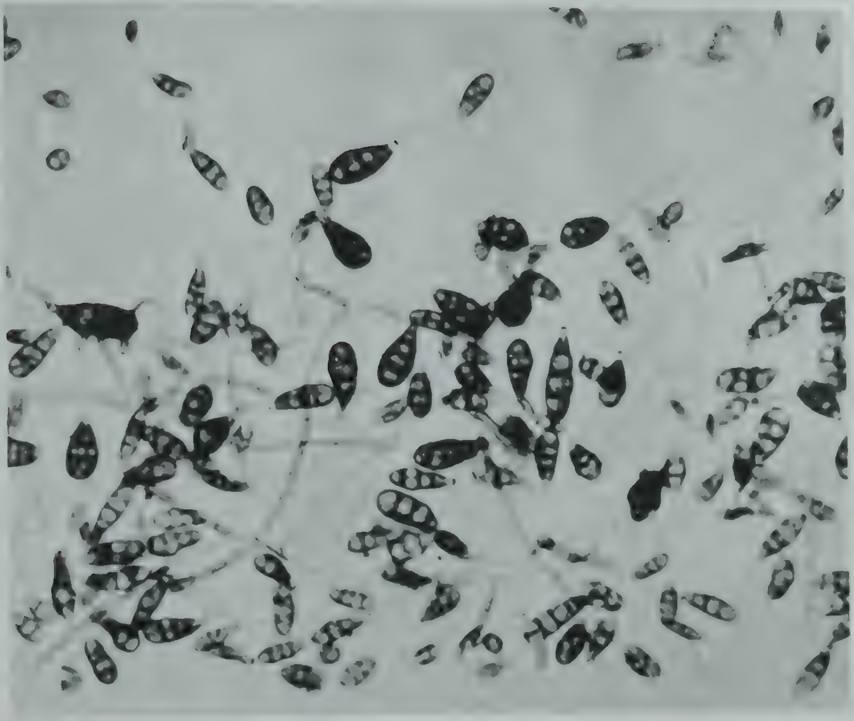

FIG. 60.—*Alternaria tenuis*—spores from culture on poor medium. × 250.

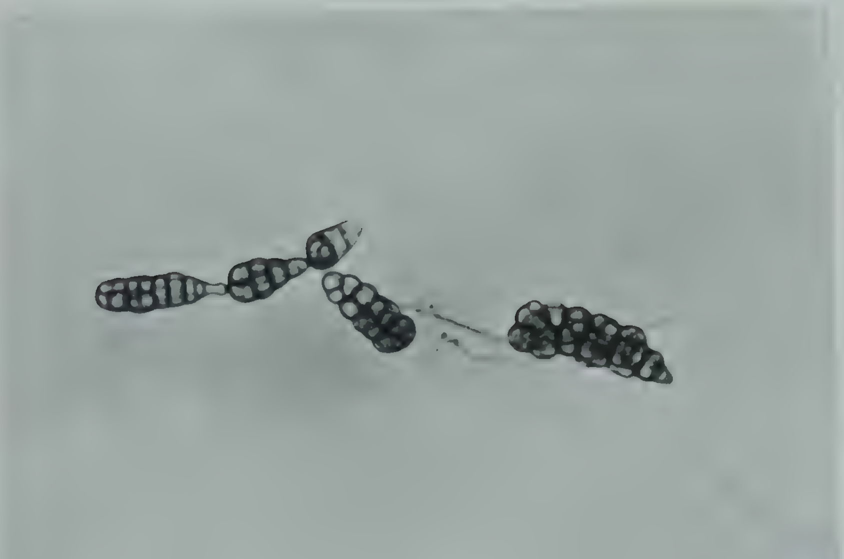

FIG. 61.—*A. tenuis*—spores from culture on rich medium. × 250.

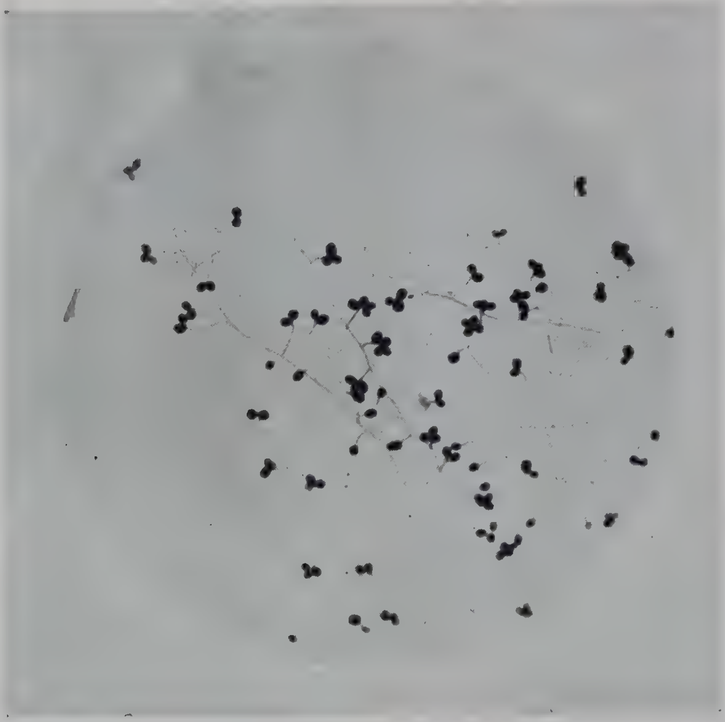

FIG. 62.—*Stemphylium sp.*—as seen in dish culture. × 65.

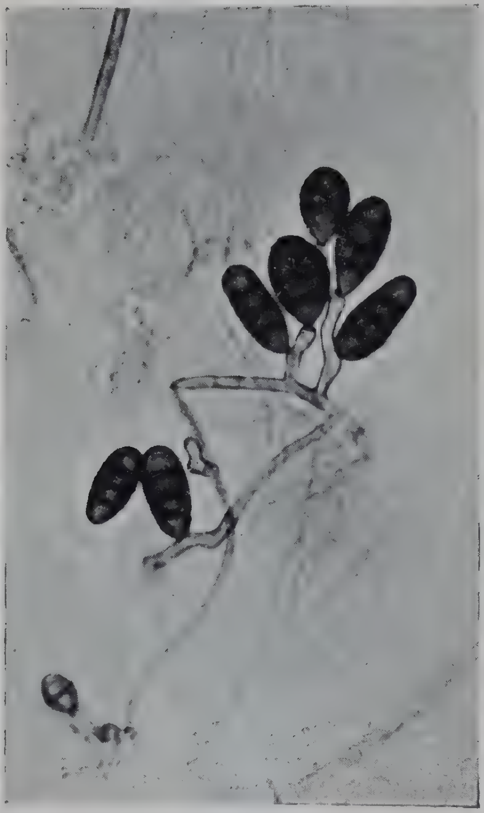

FIG. 63.—*Stemphylium sp.*—short conidiophore with cluster of spores. × 500.

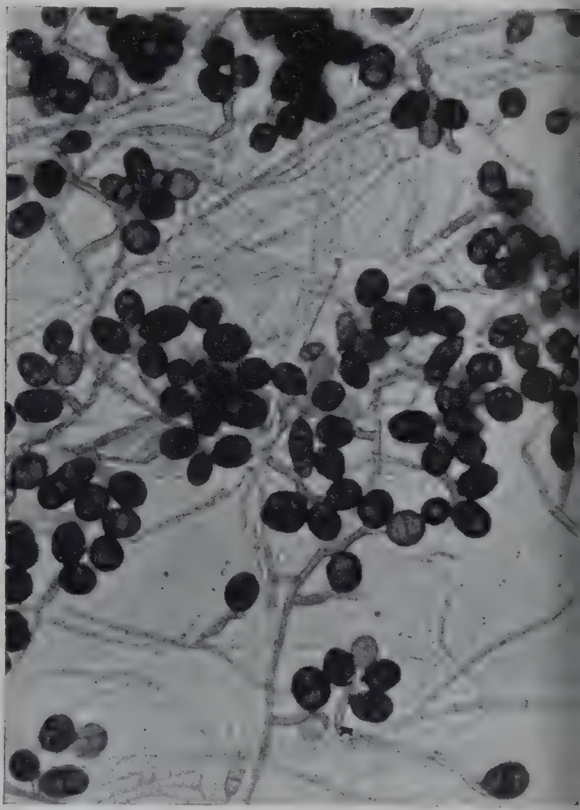

FIG. 64.—*Stemphylium sp.*—spores showing very usual type of septation. × 250.

Helminthosporium (Dem.). All of the many species of this enus are best known as aprasites of cereals and grasses. Many of iem, however, grow well as saprophytes and are not uncommon n vegetable materials. In culture some species grow normally, ith dark-coloured hyphæ and typical spores. Others appear to be uite sterile and tend to produce considerable amounts of hyaline r pale-coloured mycelium. It has been shown by Raistrick and o-workers (see Chapter XIV) that certain species of the latter roup, when grown on Czapek-Dox solution, synthesize red or purple igments which are hydroxyanthraquinones, and are closely related o the parent substances of some of the most valuable of the fast yes. In all the published keys to the Dematiaceæ *Helminthoporium* is given as producing spores singly at the ends of the conidiohores, whereas examination of a sporing culture will usually show usters of spores resembling bunches of bananas. Actually the iort, stiff conidiophore bears first a single spore slightly to one side f the tip. The tip then elongates somewhat and bears a second pore in like manner and this process may continue until a dozen or iore spores have been formed (Fig. 57). Sometimes the intervals re fairly long, and the method of their formation is readily observed, ut often the spores are closely packed and appear to be in true usters. The spores are fairly long, roughly cylindrical, often somehat attenuated at the ends, smooth, dark coloured, and appear, hen examined under low magnification, to have several to many ross septa. Actually the spores are not truly septate but contain central row of structures, termed "protoplasts" by Stevens (1922), hich have no connection with the outer wall of the spore. None the species is of appreciable importance in industrial work and ose wishing to identify them should consult the papers of Drechsler 923) and Nisikado (1929).

Heterosporium (Dem.) is similar to *Helminthosporium*, but the ores are mostly somewhat shorter and are distinctly rough. All e species are parasitic and the genus is included here merely cause occasional cultures appear when mouldy materials are plated t in the laboratory.

Alternaria (Dem.). Some species are of common occurrence on umerous kinds of organic material under damp conditions, whilst hers are parasitic on cultivated plants. In culture on rich media ost forms spread rapidly, with densely floccose, dirty green ycelium. On poor media growth is less floccose and darker in lour. The mycelium is very richly septate and often forms chains of short, swollen cells not unlike oidiospores. The conidia vary in shape from roughly ovate to inverted club-shaped, with a more or less pronounced beak at the apex. They are produced in short chains, often branched, are greenish brown to dark brown in colour and have both cross and longitudinal septa, the degree of septation increasing with age (Figs. 58, 59). On rich culture media spore production is usually sparse, but the individual spores are large and elaborately septate (Figs. 60, 61). A paper by Elliot (1917) has simplified the taxonomy of what was previously a difficult and confusing genus and should be read by those who are interested in *Alternaria* and the related genera, *Macrosporium* and *Stemphylium*. For a few species of *Alternaria* the perfect stage is known, in every case belonging in *Pleospora*.

Stemphylium (Dem.). Species of *Stemphylium* are common saprophytes, frequently being found in association with *Cladosporium* and *Alternaria*. The conidiophores occur as short branches from trailing hyphæ, bearing the spores in small clusters (Figs. 62, 63). The spores are rounded, not beaked, and usually very dark in colour. They mostly have comparatively few septa and are often sarceniform (Fig. 64).

The type species of the genus, *S. botryosum* Wallroth, differs both in shape of spores and in method of spore production from the common saprophyte here illustrated. Wiltshire (1938) is, however, of the opinion that, for the present, both types should be retained in the genus. For a full discussion of the taxonomy of *Stemphylium* and a number of related genera Wiltshire's paper should be consulted.

Coremium (Stilb.). The type species, *C. glaucum* Link, is most probably identical with *Penicillium expansum*, which, under special conditions, produces large, well-formed coremia. *C. sylvaticum* Wehmer is similarly a synonym of *P. claviforme* Bainier.

Isaria (Stilb.). Species of this genus are best known as parasites of insects, but a few forms are able to grow on ordinary culture media, where they form long white coremia, approximately cylindrical and fertile along the whole length (Figs. 65, 66).

Stysanus (Stilb.). One species, *S. stemonitis* Persoon, is encountered sufficiently often to be mentioned here. Colonies are greyish black in colour and consist chiefly of densely crowded coremia. The latter have slender stalks, and cylindrical to club-shaped heads with ovate spores about 7μ in long axis (Figs. 67, 68).

Fusarium (Tub.). Taxonomically this is the most difficult of all the genera of mould fungi. Not only is the number of species

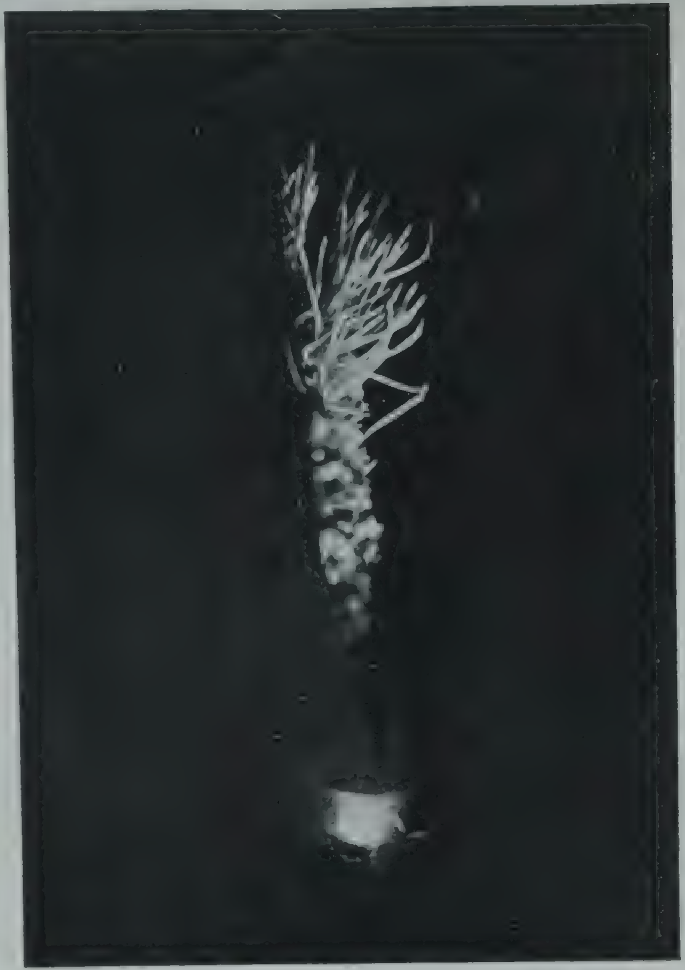

Fig. 65.—*Isaria sp.*—culture on agar slope. Natural size.

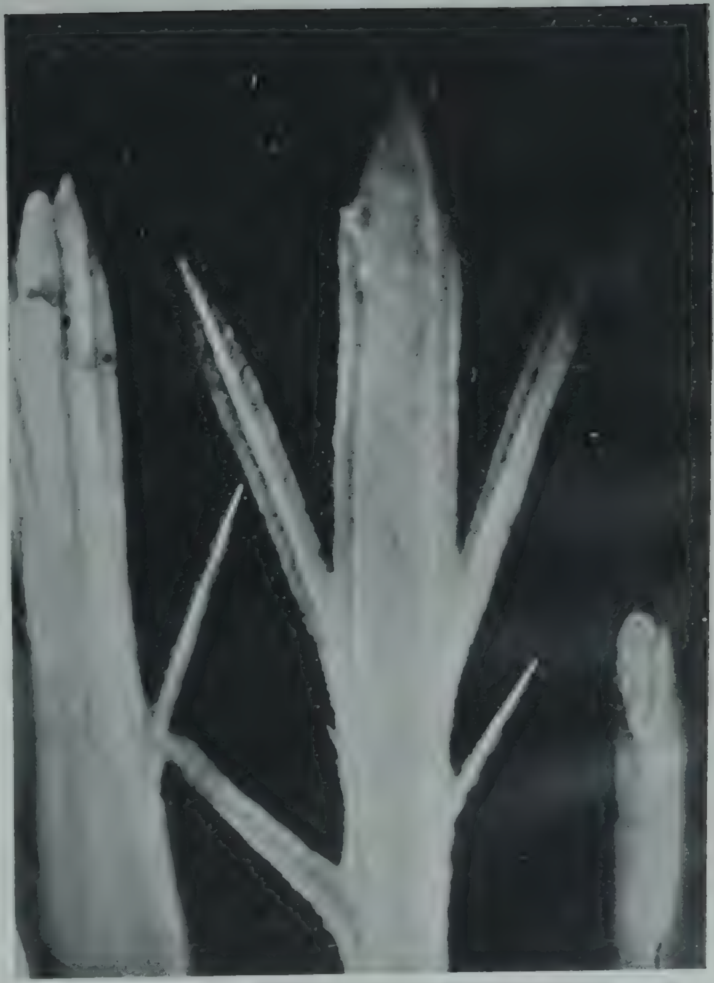

Fig. 66.—*Isaria sp.*—branched coremium. × 12·5.

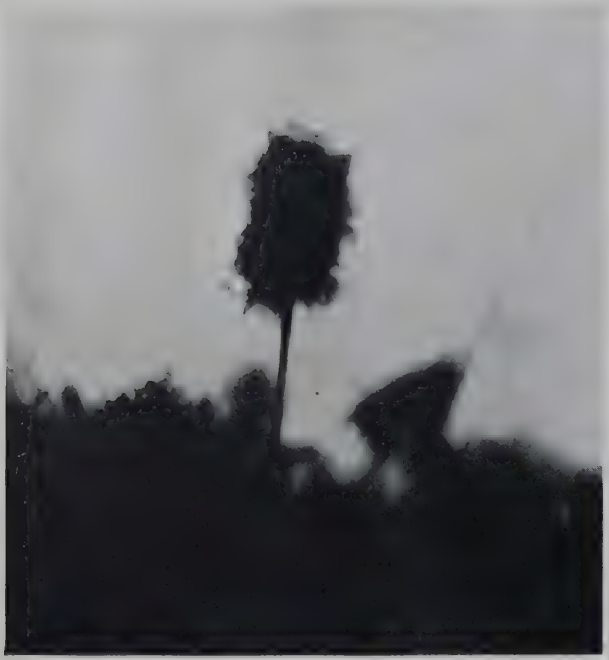

FIG. 67.—*Stysanus stemonitis*—typical coremium on agar slope. × 50.

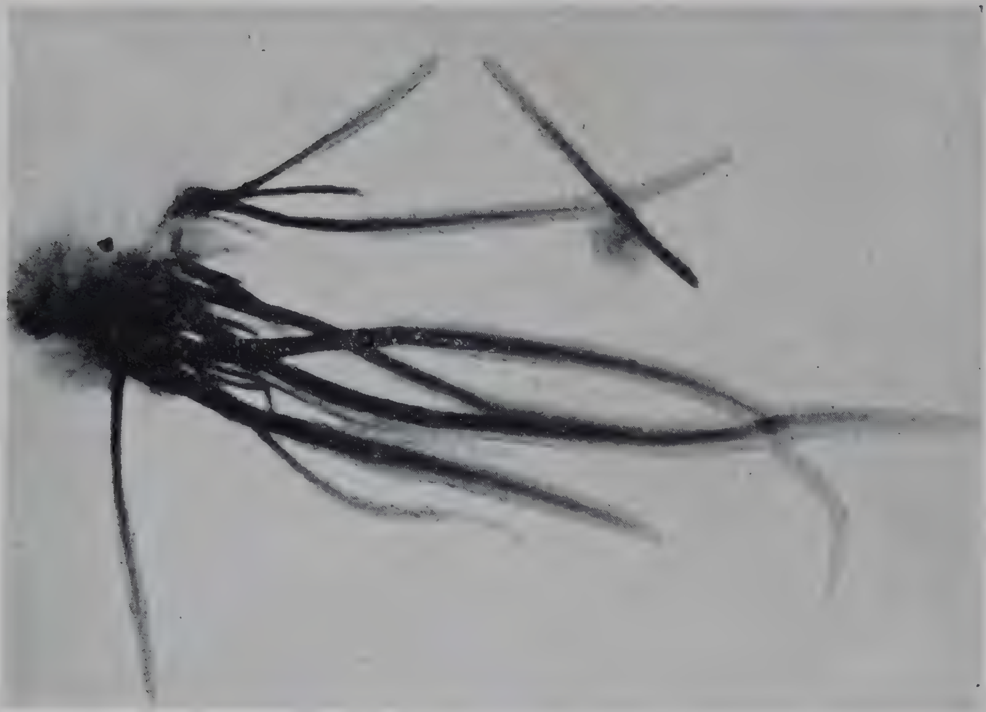

FIG. 68.—*Stysanus stemonitis*—young coremia, mounted. × 50.

very large but most species are difficult to maintain in a state of stability. Wollenweber, who has done more than any other worker to clear up the *Fusarium* problem, has stated that *Fusaria* pass through certain well-defined stages when kept in cultivation. Under favourable conditions they normally pass from a mycelial to a freely sporing stage, and thence to a degenerate state with spores abnormal and sparingly produced. Another view is that the whole question of the changes which take place can be explained by the readiness with which many species produce saltants, the relative vigour of these determining the type of growth propagated if no particular care is taken to separate the forms when sub-culturing. The latter opinion is supported by the fact, observed by many workers, that transfer of spores usually will produce sporing cultures, whilst transfer of mycelium will tend to retard spore production and give completely sterile colonies. For culture of *Fusaria* so as to retain as far as possible desired characteristics Wollenweber *et al.* (1925) recommend various media made from vegetable materials. Brown (1925), however, prefers a synthetic medium containing glucose, 2 g.; asparagine, 2 g.; K_3PO_4, 1·25 g.; $MgSO_4$, 0·75 g.; agar, 15 g.; water to 1 litre. The only disadvantage of this is that, owing to its containing only a very small percentage of total solids, it dries out quickly and shrinks to a mere streak on the glass of the culture tube if cultures are kept for more than a few weeks. The best of the common media, for maintenance of stock cultures, is ordinary potato agar.

Many *Fusaria*, when first isolated, produce mycelium alone and it is often a matter of some difficulty to induce production of normal conidia for purposes of identification. Some will spore when the cultures become old and dry, others respond to a starvation diet such as tap-water agar, whilst some seem to need a particular medium which can only be found by trial. If such mycelial cultures are kept on media containing a fairly high percentage of sugar, such as wort or Czapek agar, they tend, after several sub-culturings, to degenerate to a slimy stage, consisting of colourless, gelatinous masses of anastomosing hyphæ, and are then almost impossible to restore to the normal form.

In all systems of classification *Fusarium* is included with the Tuberculariaceæ, but many species produce, instead of sporodochia, smooth, gelatinous layers of spores (pionnotes) and are thus more nearly allied to the Melanconiales, and still others form neither sporodochia nor pionnotes, but produce conidia in scattered clusters on all parts of the aerial mycelium. The one feature common to the different types is the spore. The typical spores, or macro-conidia of *Fusarium* are sickle-shaped, with pointed ends, usually with several cross septa, colourless or pale coloured, never dark or dematiaceous, and are quite unlike those of any other mould fungi (Figs. 69, 70, 71). The exact shape, size and number of septa in the macro-conidia are of importance in determining species. In addition to the macro-conidia many species produce micro-conidia which are small, ovate, elongate, pyriform or comma-shaped, usually non-septate, more rarely 1–3 septate. A number of species form chlamydospores, which may be terminal, intercalary or both, formed in the mycelium or in the macro-spores (Figs. 72, 73). Sclerotia or sclerotial masses are not uncommon and are often brightly coloured. As in other genera, all such features have diagnostic importance.

The perfect stages of a fairly large number of *Fusaria* are now known. They are all Ascomycetes, and belong to the genera *Hypomyces*, *Gibberella*, *Nectria* and *Calonectria*.

Most of the taxonomic work on *Fusarium* has been done in connection with plant diseases and there is an extensive literature of such. The identification of species occurring as parasites is now, since the publication of the monograph by Wollenweber and Reinking (1935), comparatively easy, since it is known that the number of possible species found on any particular plant is small, and the pathological symptoms are fairly definite for each species. In industrial work, however, it is quite otherwise. Most of the *Fusaria* grow readily as saprophytes and are found in almost every situation where moulds of any kind can grow, but the difficulty of identification is still very great and, until recently, was a task for the specialist alone. In the literature of applied mycology there are numerous references to "*Fusarium sp.*" but very few, if any, references to the occurrence of particular species on industrial products. Until such information is available it would be useless to distinguish any of the species by description here.

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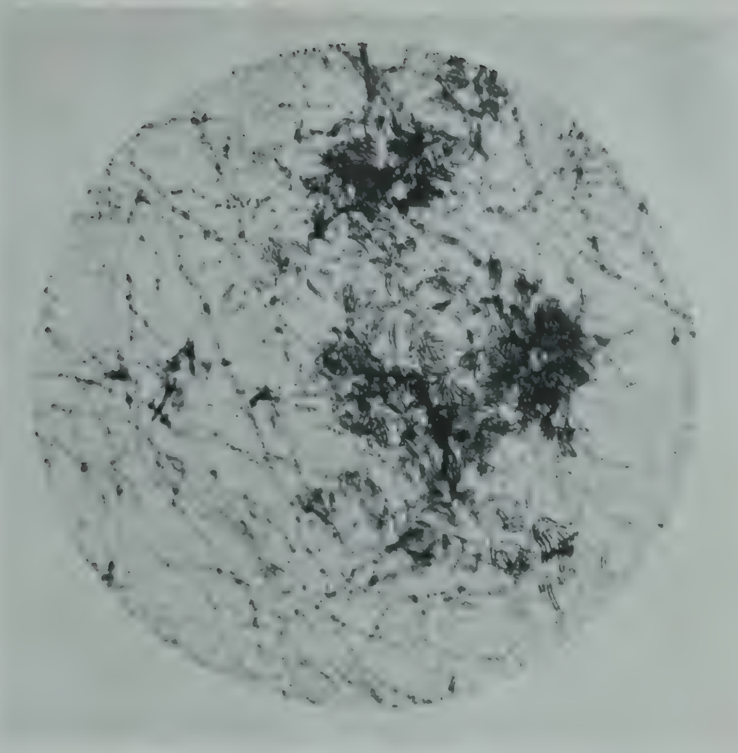

FIG. 69.—*Fusarium sp.*—clusters of conidia as seen in dish culture. × 100.

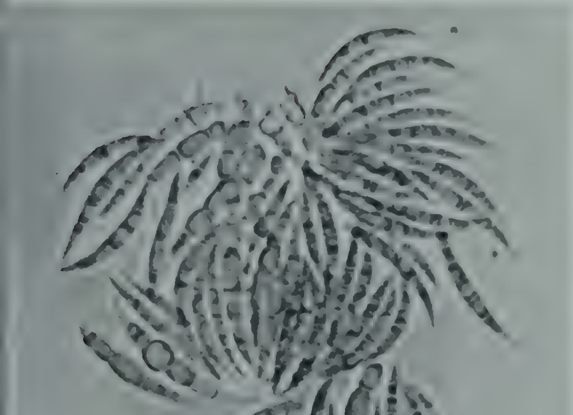

FIG. 70.—*Fusarium sp.*—cluster of conidia, a few containing chlamydospores. × 500.

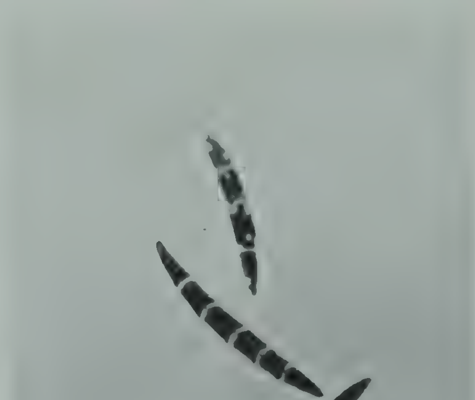

FIG. 71.—*Fusarium sp.*—typical conidia. × 1000.

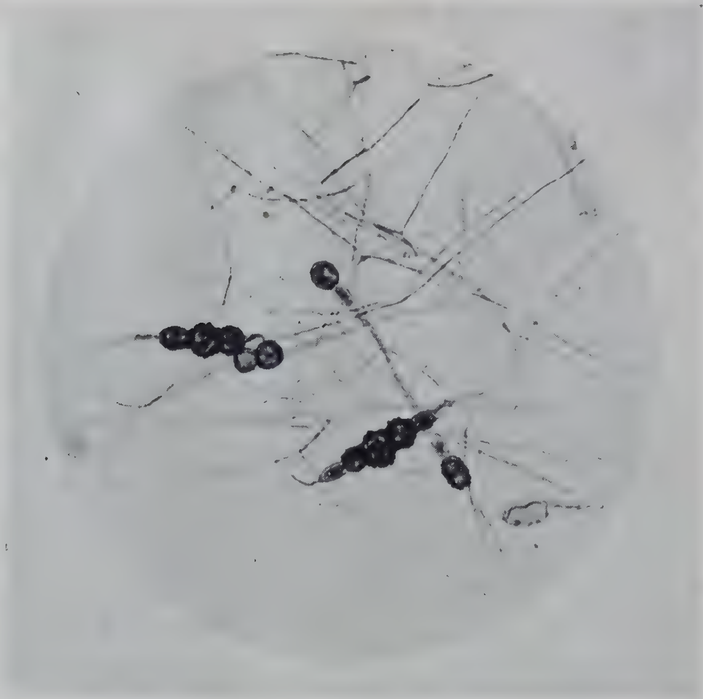

Fig. 72.—*Fusarium sp.*—chlamydospores in mycelium. × 250.

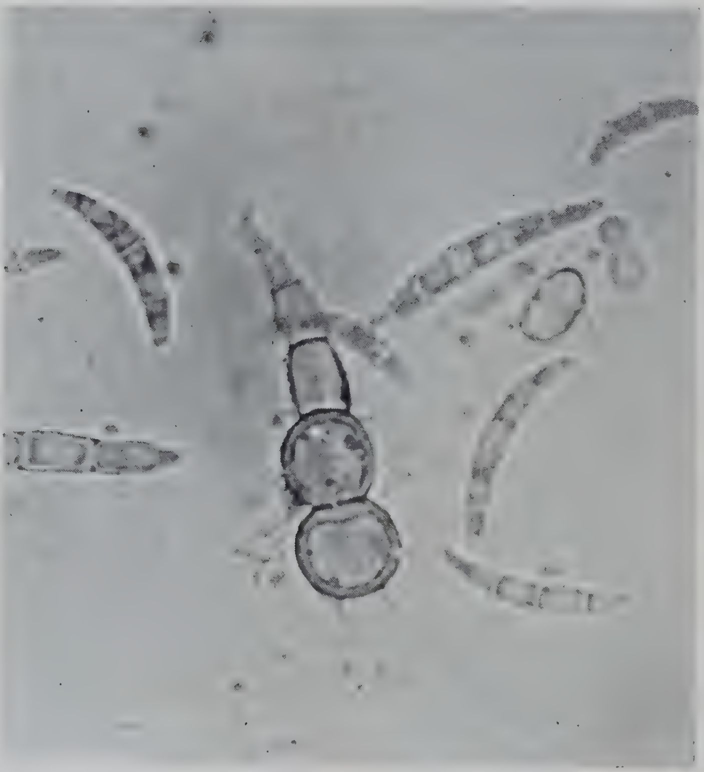

Fig. 73.—*Fusarium sp.*—chlamydospores in conidium. × 1000.

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CHAPTER VIII

ASPERGILLUS

SPECIES of this genus form a very large proportion of all the mould encountered in industrial work. They are to be found almos everywhere on every conceivable type of substratum, and a goo working knowledge of the genus is essential to anyone undertakin serious work on problems of mouldy deterioration. The activitie of the *Aspergilli*, however, are not entirely destructive in nature for several species have had their fermentative powers harnesse for commercial purposes. In the East, strains of *A. oryzœ* have lon been used for the saccharification of rice starch in the productio of saké and similar potable liquors. Strains belonging in the sam group are used in the manufacture of soy sauce and of the enzymi mixtures sold under the names “Takadiastase” and “Polyzime.’ The black Aspergilli, long known to be capable of producing considerable amounts of oxalic acid from sugar, are now used for th successful commercial production of citric acid, following Currie’s discovery that, by suitable adjustment of conditions, the formatio of oxalic acid can be inhibited and the production of the mor valuable citric acid encouraged.

The following is a generic diagnosis sufficiently broad to cover al the species of any importance.

Aspergillus. Mycelium hyaline or bright or pale coloured, o bearing surface concretions of colouring matter, never dematiaceous (black or smoky brown), septate, partly submerged and partly aerial fertile branches (stalks) arising from and more or less perpendicula to specialized, thick-walled, enlarged mycelial cells (the foot-cells), mostly non-septate, smooth, roughened or pitted, frequently enlarging towards the apex and terminating in a swelling (th vesicle) which may be variously globose, sub-globose, clavate, hemispherical, or a mere thickening of the stalk; specialized conidium-bearing cells (sterigmata) produced simultaneously, radiating from the whole or upper part of the vesicle, bearing conidia directly, or each primary sterigma bearing a cluster of secondary sterigmata, which latter then bear the conidia; conidia successively cut off by cross walls from the continuously elongating tips of the sterigmata. thus forming unbranched chains, variously shaped and coloured;

ripe spore heads of various shapes—globose, radiate, clavate or columnar—varying much in size in different species; perithecia produced by only a few species, thin-walled, breaking up to liberate the ascospores; sclerotia known in several species but not of diagnostic importance.

Some of the smaller and more delicate species bear a strong resemblance to certain of the monoverticillate *Penicillia*, but there are two criteria by which such may be separated. Thom and Church regard the presence of foot-cells as the important distinguishing feature and, in spite of the fact that Smith (1933) has described two species of *Penicillium* with true foot-cells, the criterion is quite valid for the borderline species of the two genera. Fig. 74 shows a foot-cell with the stalk and head arising from it, and this may be compared with several photographs of *Penicillia* in the next chapter. The French school of mycologists consider the method of production of the sterigmata, simultaneous in *Aspergillus* (see Fig. 75), successive in *Penicillium*, to be of primary generic significance. In the opinion of the author, both distinctions are equally to be trusted, but it is sometimes very difficult to demonstrate the presence of foot-cells in just those borderline species, owing to their compact habit and the persistence of tangled masses of mycelium in microscopic mounts. On the other hand, it is usually easy to find, especially near the edge of a growing colony, partially developed heads showing various stages in the growth of the sterigmata.

Sterigmatocystis

Various authorities in the past have considered *Aspergillus* to include only the species with one series of sterigmata, and have separated as a separate genus, *Sterigmatocystis*, all the species with two series. More recently Biourge (1933) has upheld the separation. However, Wehmer and, later, Thom and Church (1926) have rejected *Sterigmatocystis* on the ground that, in several species, the number of series of sterigmata is not constant. In particular, strains of *A. tamarii* and *A. flavus* regularly produce two kinds of heads, with one and two series of sterigmata respectively, and it is not unusual to find simple and branched sterigmata in the same head.

Eurotium

The connection of the *A. glaucus* series with the ascomycetous genus *Eurotium* has been mentioned in the chapter on the Ascomycetes. From the systematic point of view there is much to be said for retaining the name of the perfect form but, in accordance wit the consensus of modern opinion, the group of species is considere here as a section of *Aspergillus*. It should be noted that man references in the literature to this important group will be foun under the generic name *Eurotium*.

Determination of Species. The taxonomy of the genus is up to a point, not difficult owing, in large measure, to the excellen scheme of classification given in the monograph by Thom an Church. The genus, moreover, is probably unique amongst the common Hyphomycetales in the wide range of colony colou exhibited, the colours being comparatively stable and correlate with morphological and biochemical characteristics.

One of the most important factors in the clarification, by Thom and Church, of this great genus, is their conception of what may b termed the group species. Some species are fairly definite and o stable morphology. A single strain may be described in definit terms and other strains encountered will fit the description wit considerable accuracy, justifying the conclusion that here we hav a true species. In other cases, as in the series represented by *A versicolor*, *A. candidus*, *A. flavus* and others, different strains, whils clearly related, differ somewhat widely in minor details. Anyon who collects only a few strains belonging in such a series may observ differences which seem to justify the creation of several species but when scores or hundreds of strains are examined, the sharp lines o demarcation disappear and, instead of a few definite species, ther is a perfectly gradated series such that no one could describe an particular strain in terms which permit of its certain identificatio by another who had not the same series of strains before him Delimitation of species in such a group is also complicated by the fact that a single strain may cover a considerable range in the serie in response to variations in cultural conditions, or even in successiv cultures grown under the same conditions. The specialist who i interested in one particular group will collect a number of strain and will know them sufficiently well to be able to separate them o grounds dictated by his own requirements. For most purposes however, it is sufficient to be able to identify any particular cultur as a member of one of the group species. After all, this so-calle "lumping" of strains is only the quivalent of the recognition of variation amongst individuals belonging in any one species of flowerin plant or animal.

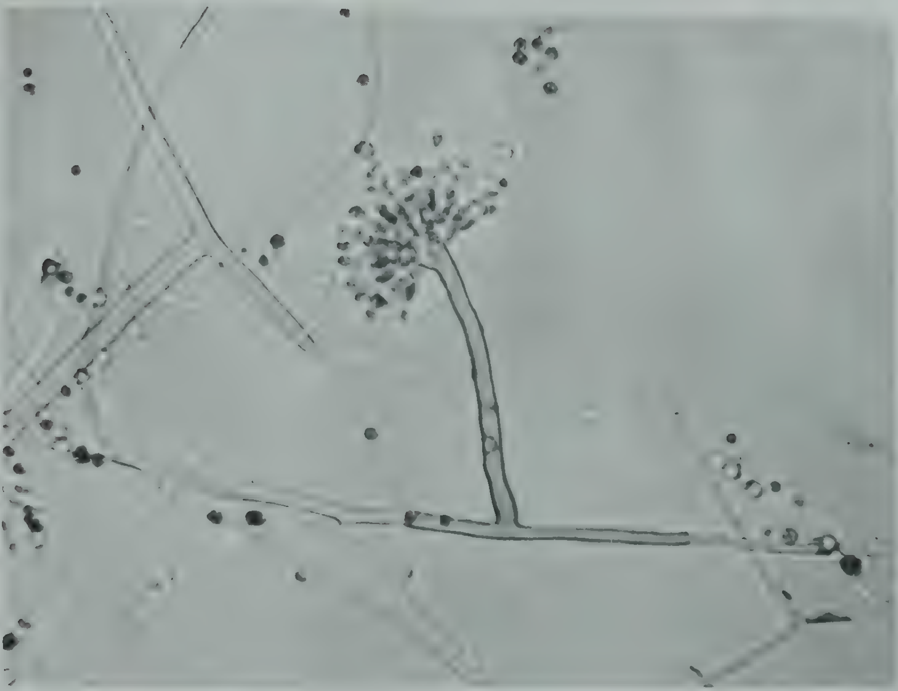


FIG. 74.—*Aspergillus ustus*—conidiophore with foot-cell. × 500.

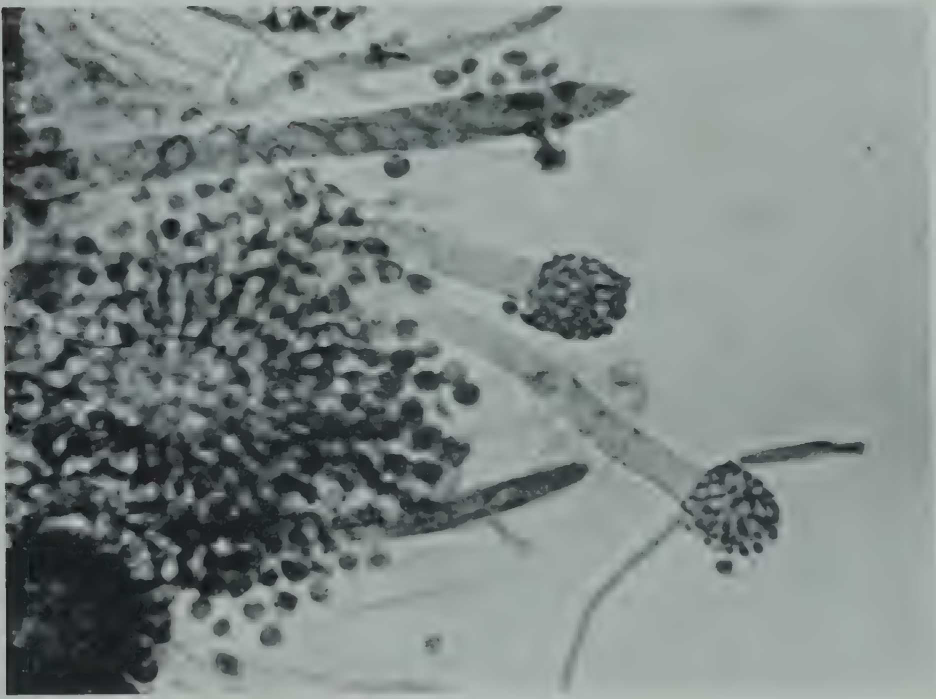


FIG. 75.—*A. repens*—showing "simultaneous" production of sterigmata. × 500.

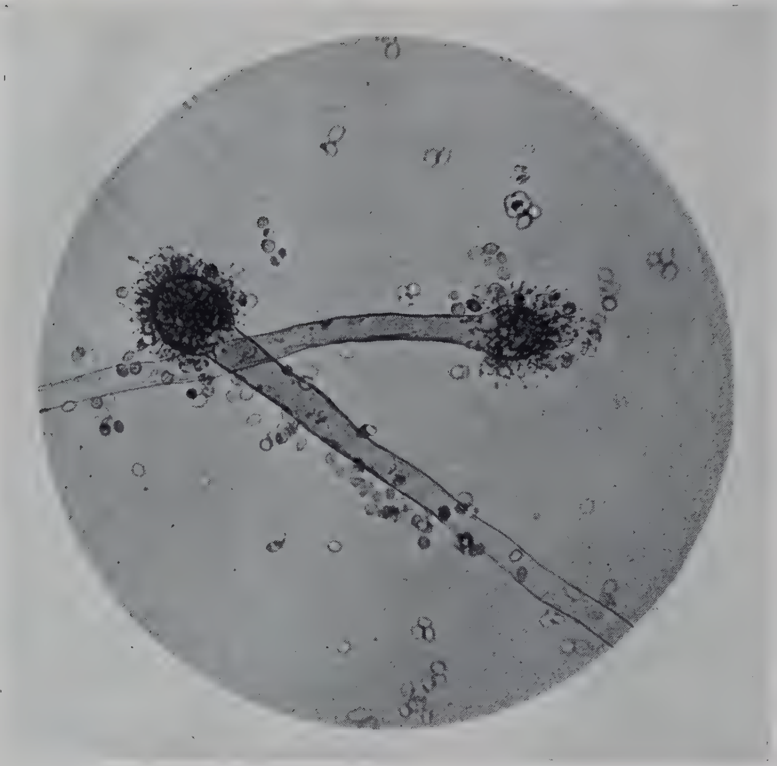


Fig. 76.—*A. glaucus*—heads with small vesicles. × 250.

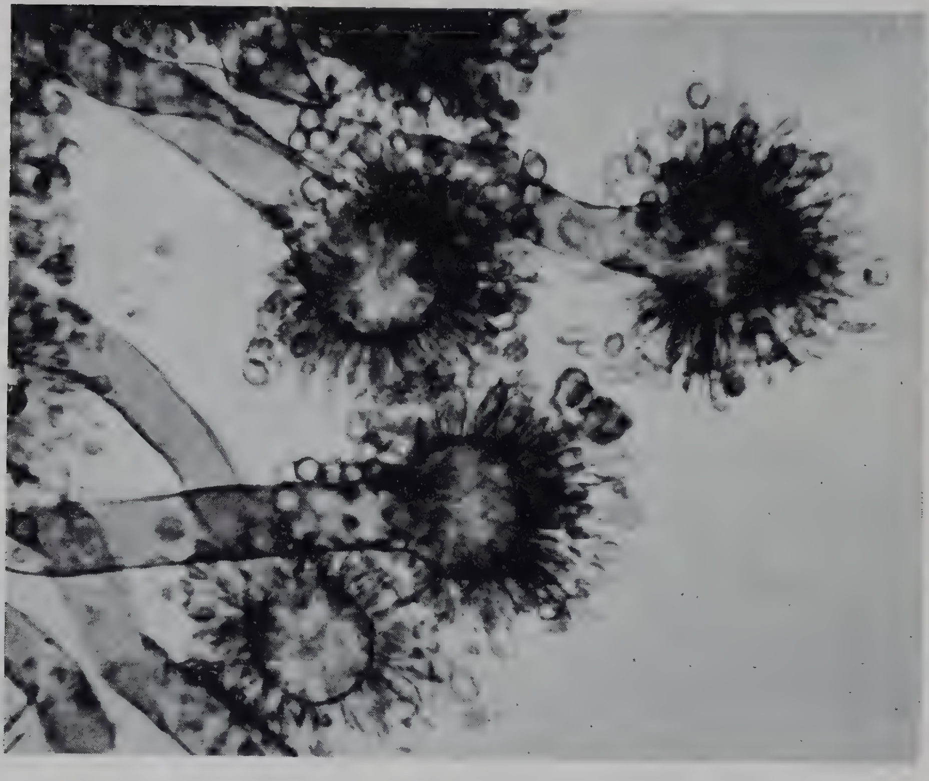


Fig. 77.—*A. glaucus*—heads with globose vesicles. × 500.

KEY TO THE PRINCIPAL SPECIES OF ASPERGILLUS

The following key is based on the classification of Thom and Church and is, for brevity, not strictly dichotomous.

1.	Stalks smooth		2
	Stalks pitted or roughened		11
2.	Vesicles elongated club-shaped	*A. clavatus.*	
	Vesicles otherwise		3
3.	Spore heads columnar		4
	Heads radiate or globose		7
4.	Colonies green		5
	Colonies sand brown, stalks colourless	*A. terreus.*	
	Colonies silvery white to pale buff, stalks yellowish	*A. flavipes.*	
5.	Sterigmata in one series		6
	Sterigmata in two series	*A. nidulans.*	
6.	Conidia, ovate, echinulate, mostly more than 4μ long	*A. penicilloides* group.	
	Conidia globose, mostly less than 4μ	*A. fumigatus.*	
7.	Conidial areas some shade of green		8
	Colonies never green		10
8.	Sterigmata in one series, conidia ovate, echinulate; yellow perithecia present	*A. glaucus* series.	
	Sterigmata in two series		9
9.	Colonies blue-green	*A. Sydowi.*	
	Colonies green or yellowish green to buff	*A. versicolor* group.	
10.	Spore heads buff to olive brown, stalks coloured	*A. ustus.*	
	Heads white to cream	*A. candidus.*	
	Heads dark brown to black	*A. niger* group.	
	Heads coffee-coloured, mycelium forming dense floccose masses	*A. Wentii.*	
11.	Heads yellow, ochre or brown		12
	Heads green, yellowish green or greenish buff	*A. flavus-oryzæ* series.	
12.	Heads bright yellow	*A. sulphureus.*	
	Heads ochraceous	*A. ochraceus.*	
	Heads greenish brown, becoming deep brown, conidia roughened by bars of colouring matter	*A. tamarii.*	

The following descriptions are believed to be sufficient for recognition of the species but, in most cases, are not complete diagnoses.

The A. glaucus Series. Species included under this name are amongst the most commonly occurring and most destructive of all moulds. They require less moisture for germination of spores and subsequent growth than practically all other moulds, or, what often amounts to the same thing, they will grow on substrata of high osmotic concentration. Thus they are commonly found, in pure culture, on jams which contains only a little less than the safe percentage of sugar, they grow on textiles which contain very little more moisture than the amount usually considered to be normal for any particular material, they attack tobacco and, in general, seem to be capable of infecting any organic material which is in equilibrium with an atmosphere of relative humidity greater than 75 per cent.

They can usually be recognized as belonging to this group—identification of species is more difficult—even in their natural habitats, and without isolation in pure culture, by the presence of both bluish green or greyish green conidial heads and bright yellow perithecia, the latter being easily visible to the naked eye.

Group diagnosis: Conidial areas mostly bright bluish green in young colonies, becoming dull green to brownish green, with the colour often partially masked by red or brown pigments encrusting the mycelium; medium frequently coloured red, brown or purple; stalks usually septate, smooth, thin-walled, often collapsing in mounts; vesicles varying from mere thickenings of the stalk (Fig. 76) to more or less globose (Fig. 77); sterigmata in one series, covering the whole or only part of the vesicle, usually broad, often proliferating to form small short stalks bearing secondary heads (Fig. 78); conidia echinulate, rarely smooth, mostly greater than 5μ in long axis, but varying much in size and attaining, particularly at low temperatures, 15μ or even 20μ, usually ovate or pear-shaped and showing a distinct connective; heads loosely radiate, having a ragged appearance when old; perithecia (Fig. 79) yellow to orange, globose or nearly so, varying from about 80μ to 250μ in diameter, containing 8-spored asci in irregular arrangement; asci usually breaking up as spores mature; ascospores hyaline, biconvex, usually with a furrow marking the line along which the wall splits in germination, with the sides of the furrow rounded or raised into crests or frills.

The group was for long a difficult one for the taxonomist. Simplification has come only after it has been recognized that satisfactory delimitation of species must be based on adequate study of a very large number of strains, and that a too narrow conception of species results only in confusion.

Mangin (1909) was the first to make a comparative study of a number of strains from various sources. He followed earlier workers in regarding size and markings of ascospores as of primary significance

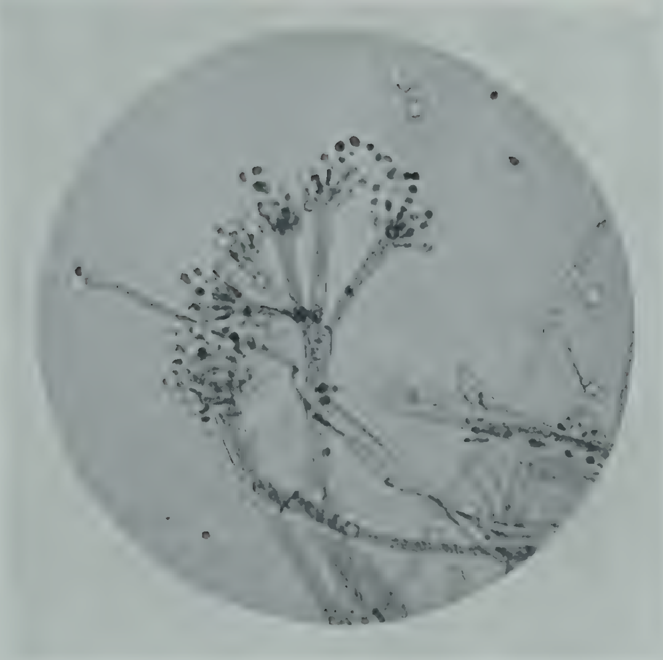


Fig. 78.—*A. glaucus*—proliferation of sterigmata to form secondary heads. × 250.

Fig. 79.—*A. ruber*—perithecia. × 100.

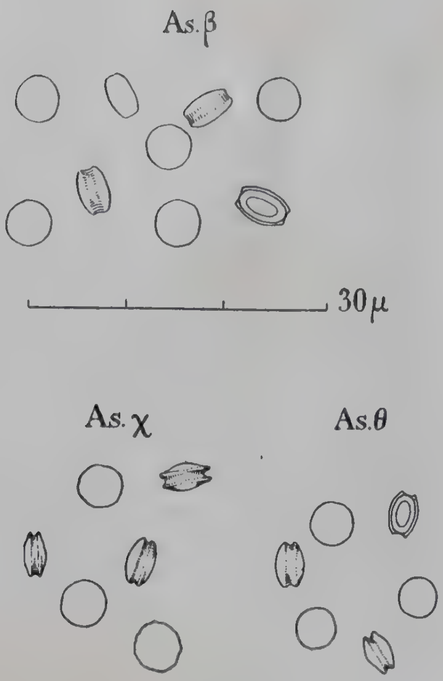


Fig. 80.—Mangin's illustration of various types of ascospore (β = *A. repens*, χ = *A. Chevalieri*, θ = *A. Amstelodami*).

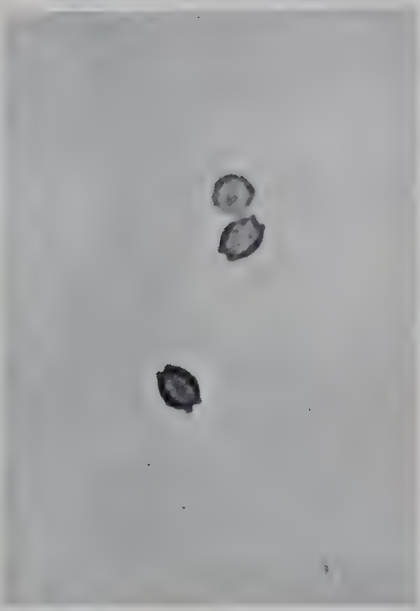


Fig. 81.—*A. Amstelodami*—ascospores as actually seen. × 1000.

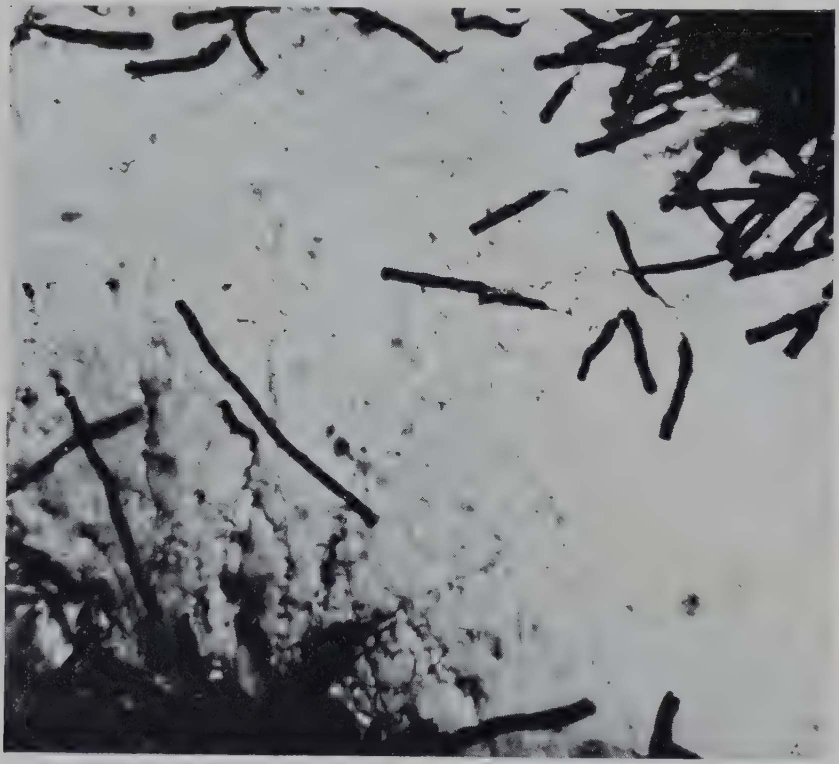


Fig. 82.—*A. restrictus*—columnar heads as seen in Petri dish. × 50.

in separating species, but the number of strains studied (23) was not sufficient for a really adequate treatment of the group. Thom and Church (1926) adopted Mangin's scheme of classification and fitted into it a number of species described by Bainier and Sartory (1911, 1912), but this section of their monograph has not proved to be entirely satisfactory in use. Recently Thom and Raper (1941) have published the results of a careful re-study of the group, and the following key to the more common species is based on their findings. It includes only the small-spores species, i.e. those with ascospores 6µ or less in long axis, since experience has shown that the large-spored forms, represented by Mangin's *A. herbariorum* series *major* and *A. herbariorum* series *minor*, are of rare occurrence.

Ascospores with curved surfaces smooth (or nearly so):

Equatorial ridges (crests) lacking; furrow nil or very shallow and indistinct *A. repens* (Corda) Saccardo.

Ridges low and rounded; furrow broad and shallow *A. ruber* (Spieckermann and Bremer) T. and C.

Ridges thin, crestlike; spore like a pulley *A. chevalieri* (Mangin) T. and C.

Ascospores with curved surfaces rough *A. amstelodami* (Mangin) T. and C.

All the species were originally included in *Eurotium* and have been transferred to Aspergillus as indicated. Fig. 80, after Mangin, illustrates his conception of the terms "crests" and "furrow."

A. repens, probably the commonest and most cosmopolitan member of the series, forms characteristic colonies, the surface being an intimate mixture of dirty green and yellow, with reverse and medium gradually becoming dirty brown on wort agar or yellow to brownish red on Czapek agar containing 20 per cent of sucrose (the medium favoured by Thom and Raper). Perithecia are mostly 75–100µ in diameter; ascospores, 4·8–5·6 × 3·8–4·4µ, smooth, with usually a trace of furrow but no crests; conidial heads large, with conidia mostly 5–6·5µ in diameter, rough.

A. ruber is also found very frequently on all kinds of substrata. Colonies are rusty red to deep red, the colour being due to incrustations of pigment on the mycelium, with reverse deep red to intense red-brown. Perithecia are usually abundant, 80–120µ in diameter; ascospores, 5·2–6·0 × 4·4–4·8µ, with broad shallow furrow; conidial heads pale blue-green turning greyish, with conidia elliptical, rough, 5–6·5µ in long axis.

A. chevalieri. Colonies show patches of pale blue-green conidial heads and areas of orange hyphæ in which perithecia are embedded, with reverse orange-red. Perithecia are 100–140μ in diameter; ascospores smooth walled, with thin, prominent crests, 4·6–5·0 × 3·4–3·8μ; conidial heads radiate, fairly large, with conidia almost globose, spinulose, 4·5–5·5μ in diameter.

A. amstelodami. Colonies are usually of a deep rich green, speckled with sulphur yellow, with reverse almost colourless or pale yellow. Perithecia are up to 140μ or even 160μ in diameter; ascospores rough all over, with prominent furrow and rounded crests (Fig. 81); conidial heads radiate or more or less columnar, large; conidia subglobose, delicately roughened, mostly about 4μ in diameter. Occasional cultures vary from type in being almost entirely perithecial, with conidial heads produced tardily and sparingly.

The A. penicilloides Group. Species belonging in this group are of common occurrence but are easily missed in plates from infected material owing to their very slow rate of growth, even on rich media. Some of them have been described as species of *Penicillium* and certainly bear a striking resemblance to certain of the monoverticillate members of that genus; hence the name. In the author's experience they are amongst the most resistant of all moulds to the inhibiting effect of antiseptics. They are easy to recognize by their restricted growth and their very long, slender, columnar heads, composed of chains of fairly large, ovate to barrel-shaped, echinulate conidia. They resemble *A. fumigatus* in disposition of sterigmata and shape of heads, and the *A. glaucus* series in size and markings of spores.

Several species have been described, but recognition of their importance has been tardy and no one has yet made a really adequate study of the group. For the time being, the following species may be distinguished:

Young colonies pale bluish green, spore chains forming compact columns, conidia slightly roughened, 4–5μ by 3μ	*A. gracilis* Bainier.
Young colonies dark green, heads globose when young, becoming columnar, conidia very dark coloured, 3·5–5μ by about 3·5μ	*A. penicilloides* Spegazzini.
Young colonies dull green, heads in long compact columns, conidia hyaline and cylindrical when young, becoming pyriform and brownish green, 4·5–6μ by 3–4μ	*A. restrictus* G. Smith.

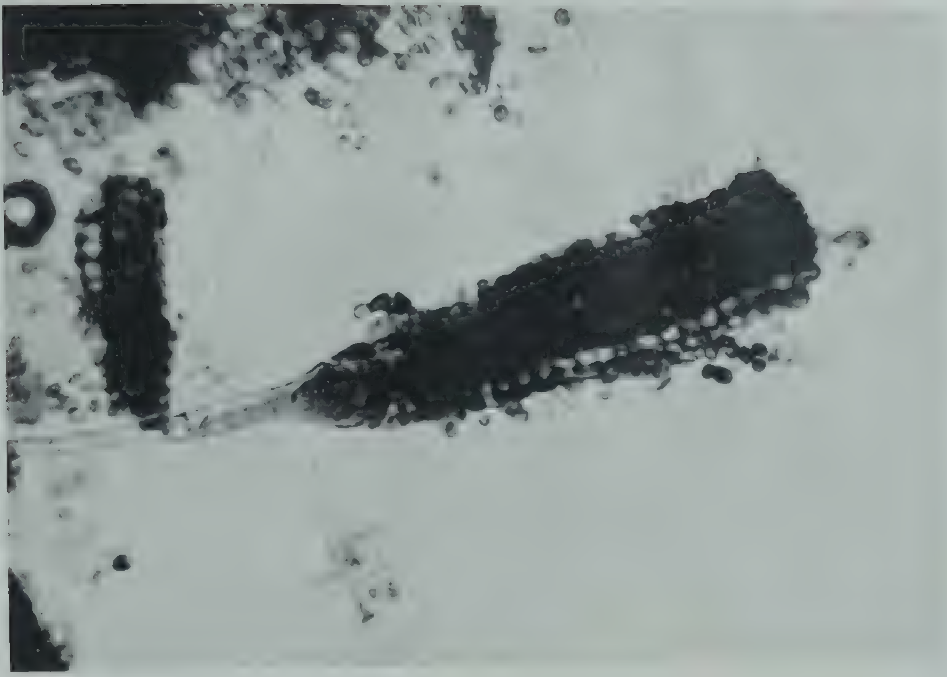


FIG. 83.—*A. restrictus*—typical conidiophore. × 500.

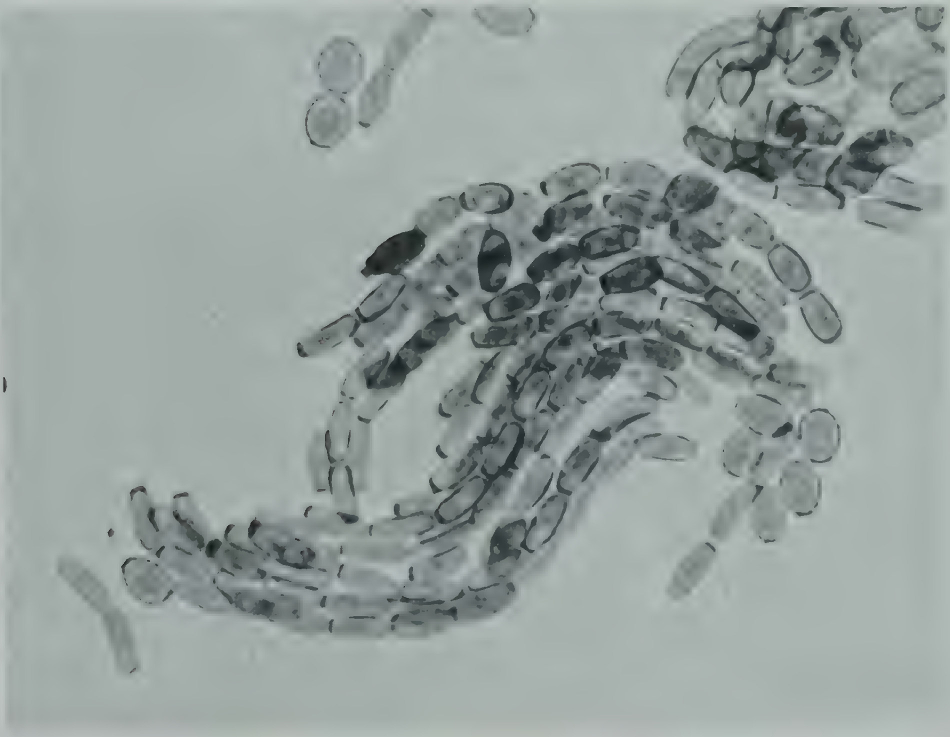


FIG. 84.—*A. restrictus*—spores, mostly young but with a few older ones showing roughening. × 1000.

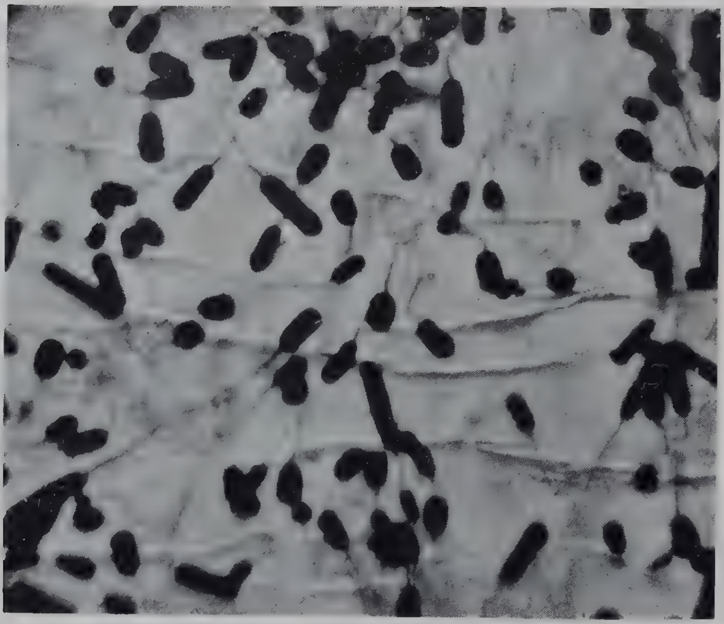


FIG. 85.—*A. fumigatus*—columnar heads as seen in Petri dish. × 50.

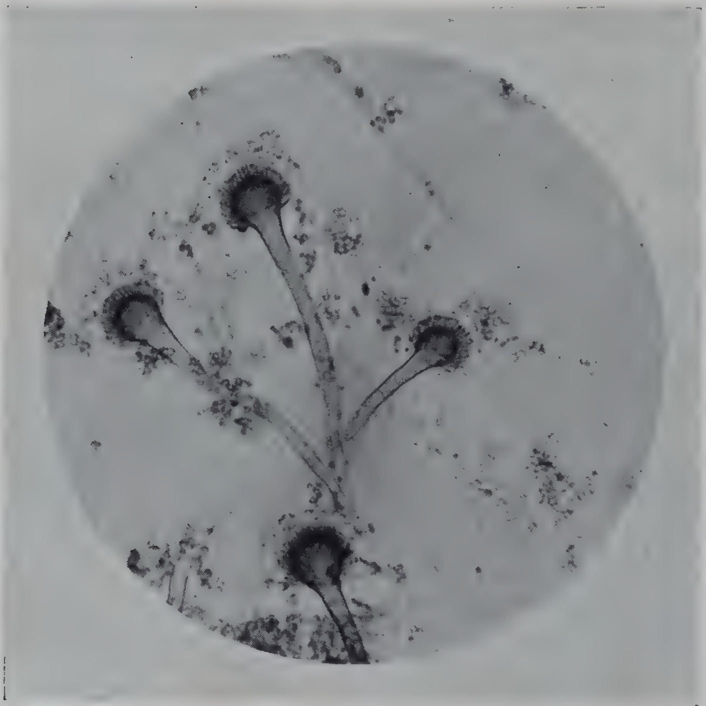


FIG. 86.—*A. fumigatus*—typical conidiophores. × 250.

Young colonies dark green, heads more
 or less columnar, enveloped in slime,
 conidia 4–6μ by 3–3·5μ . . . *A. conicus* Blochwitz.

Figs. 82, 83, and 84 show *A. restrictus*, a form which has been received from a number of workers in different parts of the world and which appears to be fairly common.

A. fumigatus Fresenius. Colonies usually dark smoky green, becoming still darker in age, and more or less velvety, in occasional strains definitely floccose, sporing tardily and with young heads a very bluish green; conidial heads columnar, varying in length and about 40μ broad (Fig. 85). Heads mounted in lacto-phenol have a very characteristic appearance (Fig. 86). The species is readily distinguished from the last group by its globose conidia, 2·5–3μ in diameter, and by its much more rapid growth. A number of perithecial strains, with the conidial morphology of *A. fumigatus*, have been described but are not of common occurrence.

This species is the causal agent of a disease of birds, known as Aspergillosis. Cases in which human beings have contracted the disease, which has many of the symptoms of tuberculosis, are rare. Nevertheless, care should be taken, when handling cultures, not to distribute spores in the air.

A. clavatus Desmazières. Colonies grow rapidly, forming a dense felt, greyish green in colour. The most typical feature of the species is the elongated, club-shaped vesicle, bearing short, densely packed sterigmata in one series over its whole surface (Fig. 87). It is sometimes possible to find, in ordinary cultures, mature heads which are definitely clavate, but mostly the chains of conidia form dense masses of irregular shape. Large club-shaped heads, on coarse stalks, can be obtained by growing on special media. The conidia are elliptical, 3·5–4·5μ by 2·5–3μ.

A closely related species, *A. giganteus* Wehmer (Figs. 88, 89, 90), differs little from *A. clavatus* when grown on ordinary media but produces enormous heads, on stalks several centimetres long, under special conditions of culture. Thom and Church specify organic nitrogen in the medium whilst Biourge (1933) states that typical gigantic heads are formed only when the medium contains zinc and the cultures are exposed to light.

A. nidulans (Eidam) Winter. Two types of culture are met with, the difference depending on the presence or absence of perithecia. Purely conidial strains form smooth, velvety colonies of a beautiful clear green colour, with reverse deep red to purple. When perithecia are being produced the green colour is confined to small areas, the rest being dirty white and floccose. The conidial heads are similar to those of *A. fumigatus* except for the presence of two series of sterigmata (Fig. 91), with stalks mostly brownish and sinuous. Reduced heads are common, consisting of very small vesicles and few sterigmata, or even single sterigmata on short stalks. Production of perithecia is uncertain and spasmodic. Some strains produce the perfect stage with fair regularity, others are invariably conidial, whilst still others give the imperfect form alone through a series of cultures and then suddenly, and for no apparent reason, produce abundant perithecia. The perithecia (Fig. 92) are more or less globose, dark red, with brittle walls, and are surrounded by masses of thick-walled cells, known as Hülle cells, up to 25μ in diameter. The ascospores are red to purple, ovate, about 5μ by 4μ.

Thom and Raper (1939) have described five species and two varieties which all have the general characteristics of *A. nidulans*, but which differ in markings and size of ascospores. The majority of strains encountered, however, all belong to *A. nidulans* proper.

A. versicolor (Vuillemin) Tiraboschi. This common organism is of world-wide distribution and is found in much the same situations as species of the *A. glaucus* series, but requires slightly more moisture than the latter for growth. As the name implies, cultures show a considerable range of colour. Different strains may be variously pale green, greyish green, buff, dirty white or even pink in small areas, whilst a single culture, on occasion, may show patches of yellow, pink, white and green on the surface, and a deep red or plum colour in reverse. The texture of the colony may be velvety, floccose, or both in patches. The heads are loosely radiate, with sterigmata in two series and small, globose, delicately roughened conidia (Fig. 93). Whatever the colour of the colony, the spores turn a beautiful emerald green when mounted in lacto-phenol.

A. Sydowi (Bainier and Sartory) Thom and Church. This species is very similar in structure to *A. versicolor* (see Fig. 94), but forms velvety colonies of a deep bluish green or greenish blue colour, with reverse usually very deep red. It is easily mistaken for a *Penicillium* at first sight and until examined under the microscope. Dwarfed and reduced heads are frequently found.

A. terreus Thom. The usual colony colour is sand brown and the surface may be smooth velvety or moderately floccose. The medium is rapidly coloured deep yellow, becoming, especially in the presence of zinc, dirty brown. Fig. 95 shows the typical columnar heads as

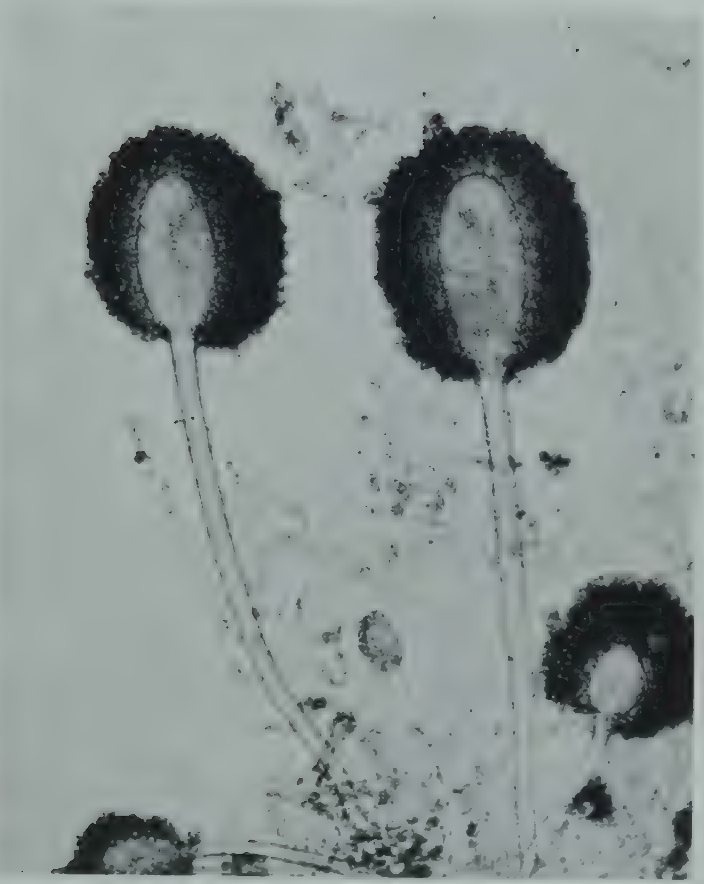


FIG. 87.—*A. clavatus*—conidiophores. × 100.

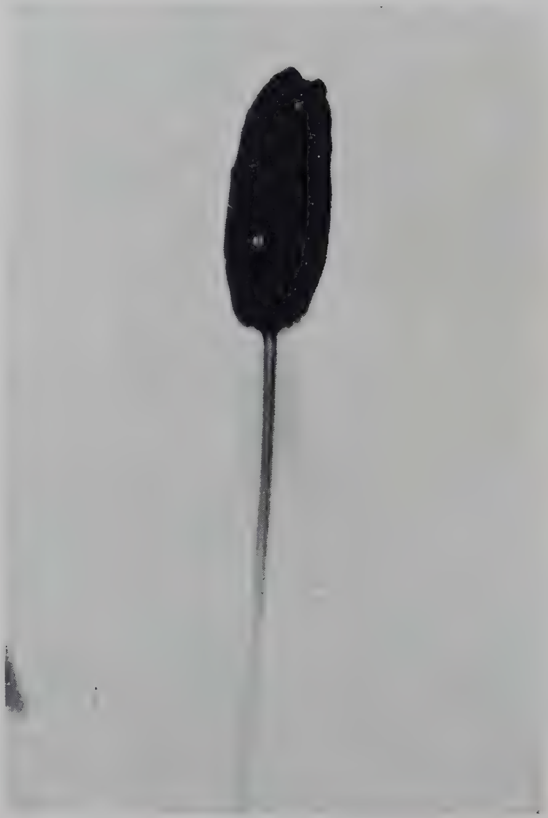


Fig. 88.—*A. giganteus*—single head of characteristic shape as seen in living culture. × 25.

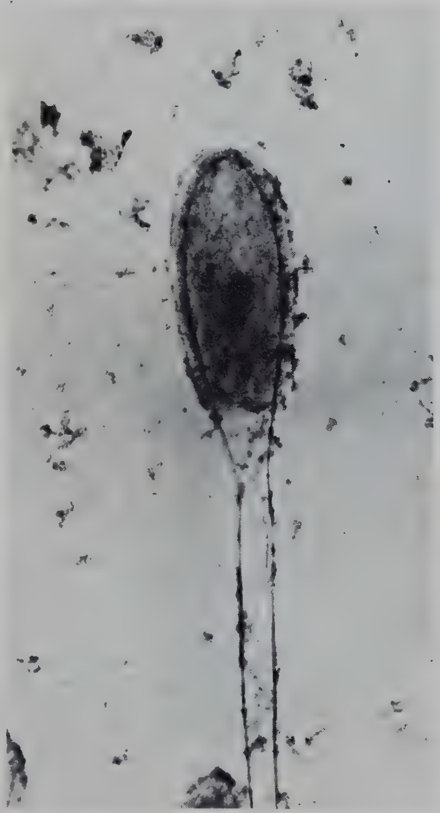


Fig. 89.—*A. giganteus*—head with clavate vesicle. × 100.

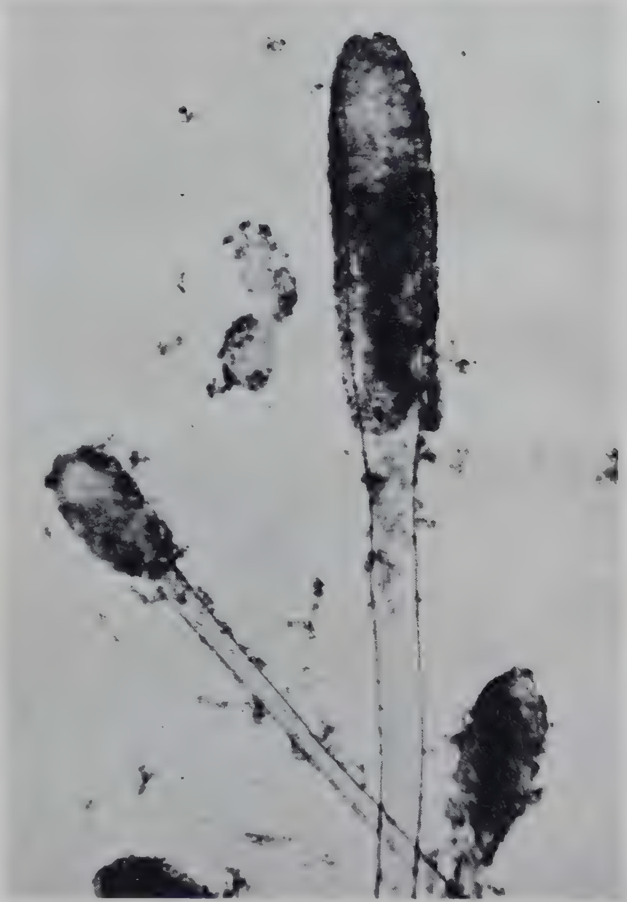


Fig. 90.—*A. giganteus*—head with elongate vesicle. × 100.

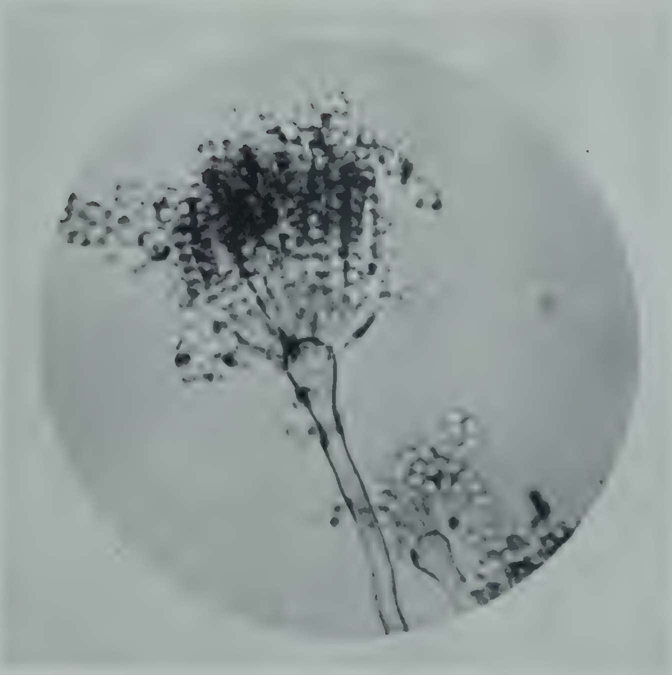


Fig. 91.—*A. nidulans*—head with columnar mass of spores (the spores have remained *in situ* much better than in most mounted specimens). × 250.

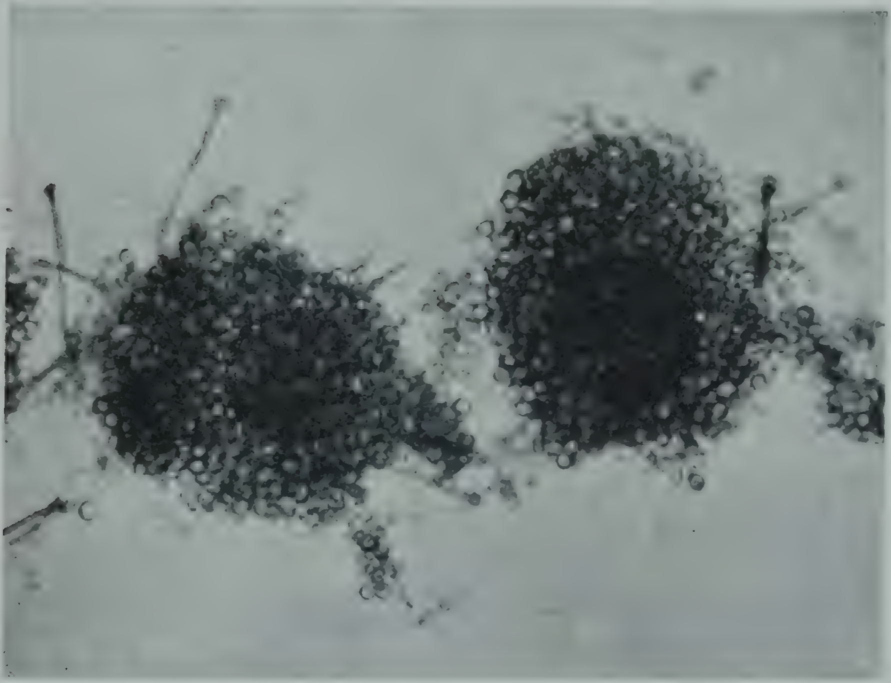


Fig. 92.—*A. nidulans*—perithecia with masses of Hülle cells. × 100.

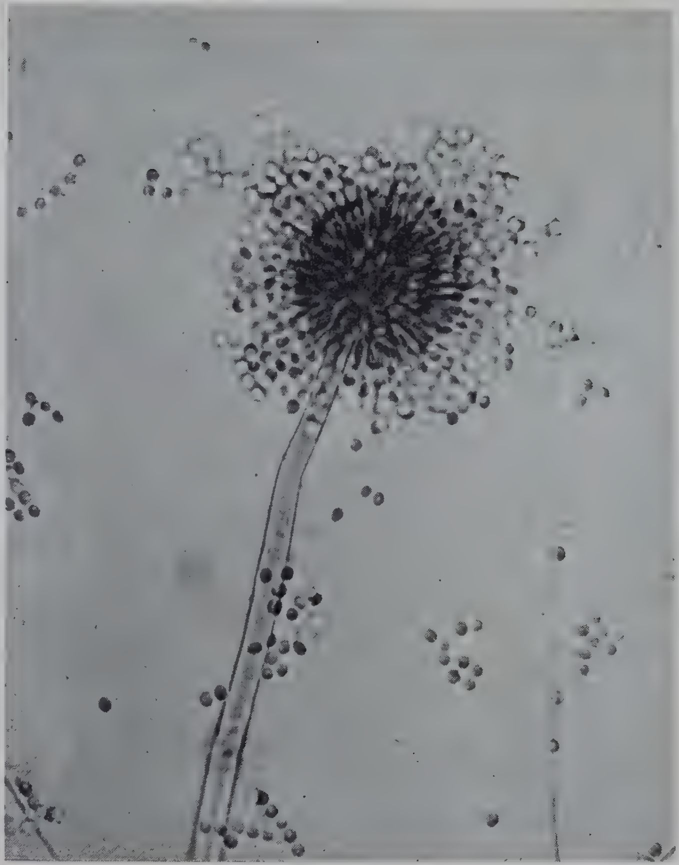


Fig. 93.—*A. versicolor*—typical head. × 500.

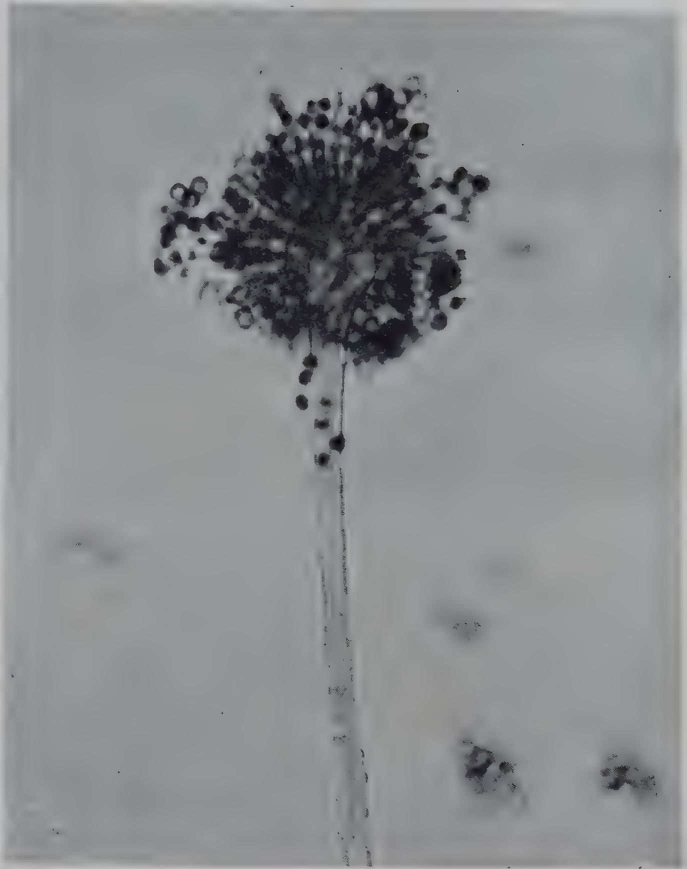


Fig. 94.—*A. Sydowi.* × 500.

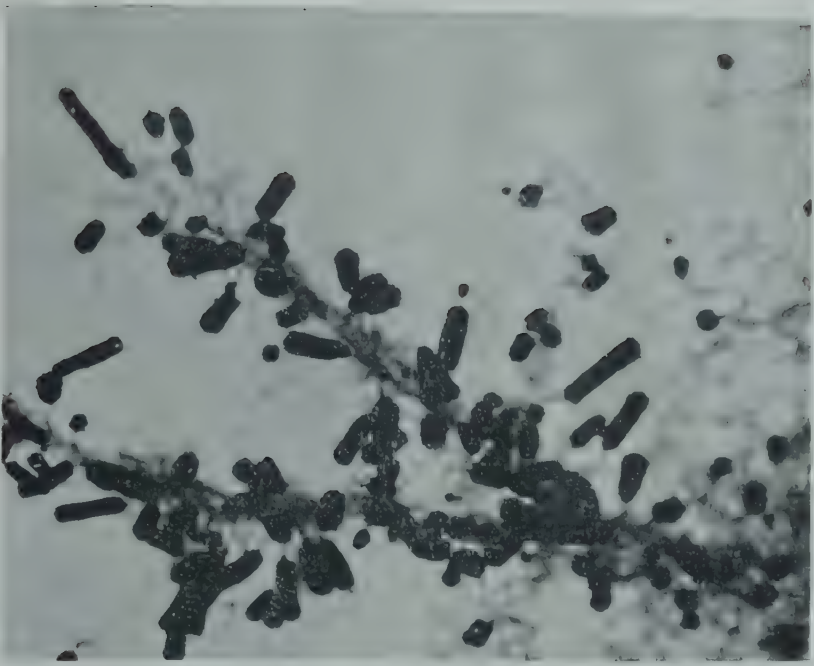


FIG. 95.—*A. terreus*—columnar heads as seen in Petri dish. × 50.

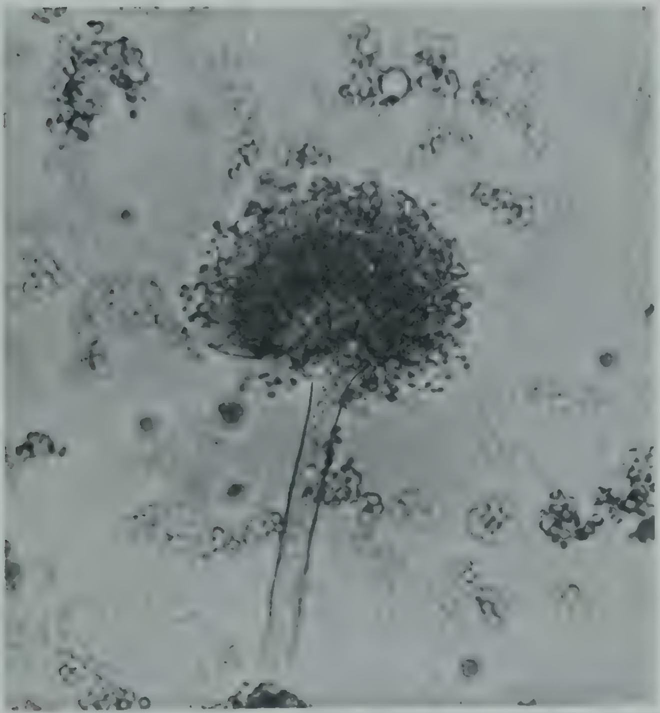


FIG. 96.—*A. terreus*—head with dome-shaped vesicle. × 500.

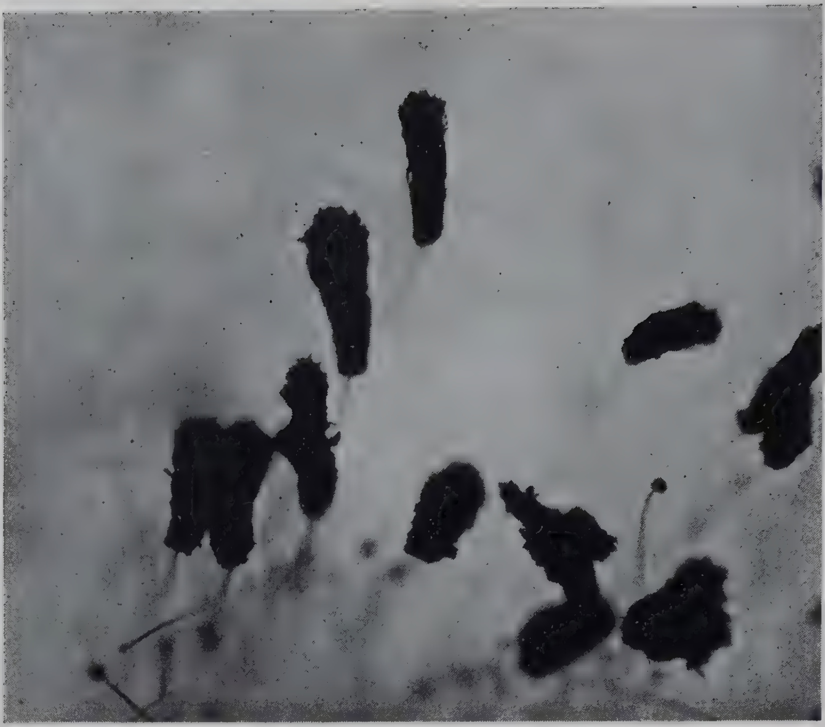


Fig. 97.—*A. flavipes*—columnar heads as seen in old culture. × 50.

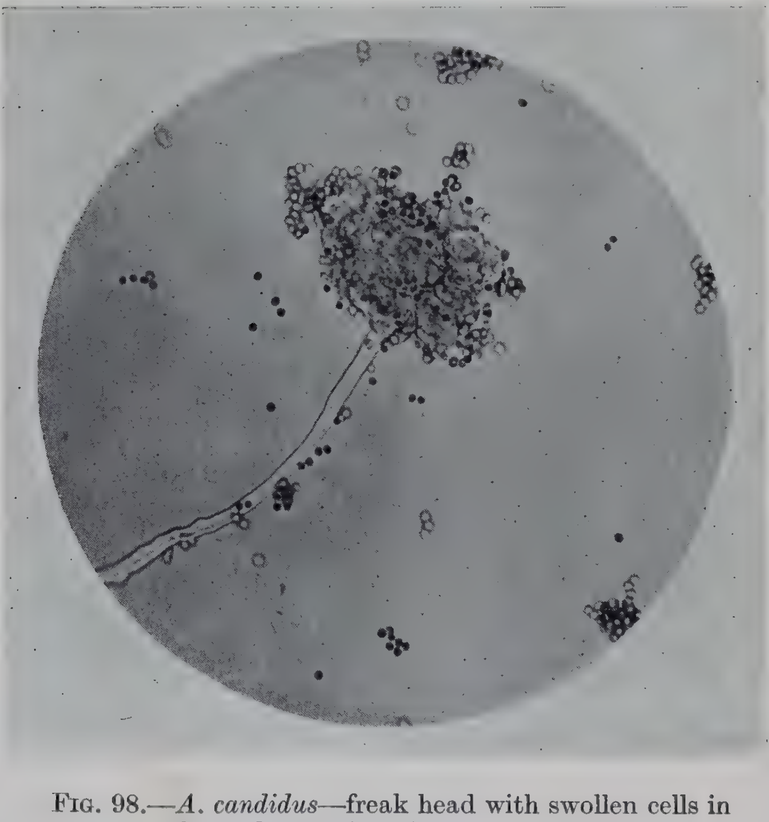


Fig. 98.—*A. candidus*—freak head with swollen cells in place of normal sterigmata. × 300.

een in a living culture and Fig. 96, taken from a mounted specimen, he shape of the vesicle and disposition of the sterigmata. *A. terreus* s a common soil organism and is fairly frequently encountered on ll types of vegetable material. The author has found it on scores of samples of Egyptian cotton.

A. ustus (Bainier) Thom and Church. Colonies are floccose, at irst white, then brownish yellow and finally brownish grey, with reverse yellow, dull reddish or purplish. The stalks are short, brownish, smooth and 5–10μ in diameter. The vesicles are globose, 10–20μ diam., fertile over the upper two-thirds. Sterigmata are n two series, primary 5–6μ and secondary 6–9μ long, and the conidia globose, spinulose, 3–3·5μ diam. Perithecia are unknown but Hülle cells are found in some strains, being globose, ovate or sausage-shaped (cf. *A. nidulans*).

A. flavipes (Bainier and Sartory) Thom and Church. The usual type of colony is white or silvery white, sometimes with patches of pale pink or mauve, with the white colour persistent or changing to pale buff or greyish buff. Some strains produce brown sclerotia. The buff forms, in old culture, often approach the appearance of *A. terreus*, but the two are readily distinguished by examination of young, vigorously growing colonies. The white strains are not likely to be confused with the *A. candidus* group owing to the difference in shape of the heads. Stalks are yellow or brownish yellow; the heads, at least in old cultures, definitely columnar (Fig. 97); the sterigmata in two series; the conidia smooth, globose, 2–3μ in diameter.

A. candidus Link. The name covers a number of somewhat diverse forms with white or creamy white globose heads. Most strains grow slowly, producing scanty mycelium and glistening white heads, varying from about 100μ to 250μ in diameter, borne on erect, colourless stalks up to 1 mm. in length. The vesicle is globose and bears two series of sterigmata over the whole surface. Occasionally freak heads are found, with curious swollen cells in place of the usual slender sterigmata (Fig. 98). The spores are globose, smooth, colourless, 2·5–3·5μ in diameter. Some strains produce purple sclerotia, but such are not common.

The A. niger Series. Black *Aspergilli* are of very common occurrence everywhere and, as is usual in any group of widespread distribution and omnivorous habits, there are a large number of races, strains or species. The following group diagnosis will make clear the type of variation to be expected.

Colonies spreading rapidly, with mycelium white at first, frequently developing areas of bright yellow; stalks arising from the substratum, varying from 200μ to several millimetres in length and from about 10μ to 20μ in diameter, mostly colourless, thick-walled, smooth; heads globose, often splitting at the periphery (Fig. 99); vesicles globose, colourless or yellowish brown, fertile over the whole surface, up to about 50μ in diameter; sterigmata in one series in a few strains but usually in two series, with the secondaries fairly uniform in size, about 7μ by 2–3μ, and the primaries varying in length from 10–15μ in *A. niger* to 120μ in *A. carbonarius*, both series usually deep brown; conidia globose, brown to black or purplish brown, with the colour aggregated into bars and nodules between the inner and outer walls, appearing spinulose except at very high magnification, varying from 2·5μ to 10μ in diameter. Fig. 100 shows a typical head after decolorization. Sclerotia are sometimes found in freshly isolated strains, these being round, white, and about 1 mm. in diameter, but it is usually found that such strains become purely conidial after a few transfers.

The dimensions of primary sterigmata and of conidia are made the basis of separation into a number of fairly definite species. The following table is after Thom and Church, omitting one or two doubtful species:

Sterigmata in one series:	
A. luchuensis Inui . . .	Conidia 4–5μ, sterigmata 7–9μ long.
Sterigmata in two series:	
A. luteo-niger (Lutz) T. and C. .	Conidia about 5μ, with colour bars dissolving in fluid mounts.
A. niger van Tieghem . .	Conidia 2·5–4μ, primary ster. 20–30μ.
A. phœnicis (Corda) T. and C. .	,, 3–4μ, ,, ,, 40–60μ.
A. pulverulentus (McAlpine) T. .	,, 3–4μ, ,, ,, 120μ.
A. atropurpureus Zimmermann .	,, 6–8·5μ, ,, ,, 40μ.
A. carbonarius (Bainier) Thom	,, 8–10μ, ,, ,, 120μ.

It is hardly surprising that, with a species of such widespread and common occurrence, mutations have from time to time been obtained. Thom and Church mention several stable mutants obtained experimentally by growing *A. niger* on media containing small amounts of poisonous substances, the most distinctive of these being *A. cinnamomeus* Schiemann, almost colourless, and *A. Schiemanni* (Schiemann) Thom, with brownish colonies. More recently Yuill and Yuill (1938) have described as *Cladosarum olivaceum*

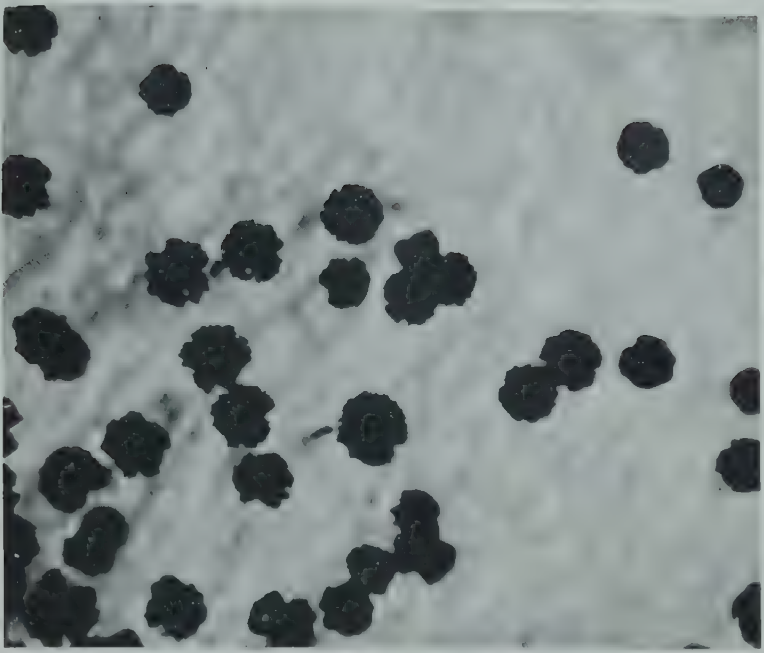


Fig. 99.—*A. niger*—globose heads as seen in Petri dish. × 20.

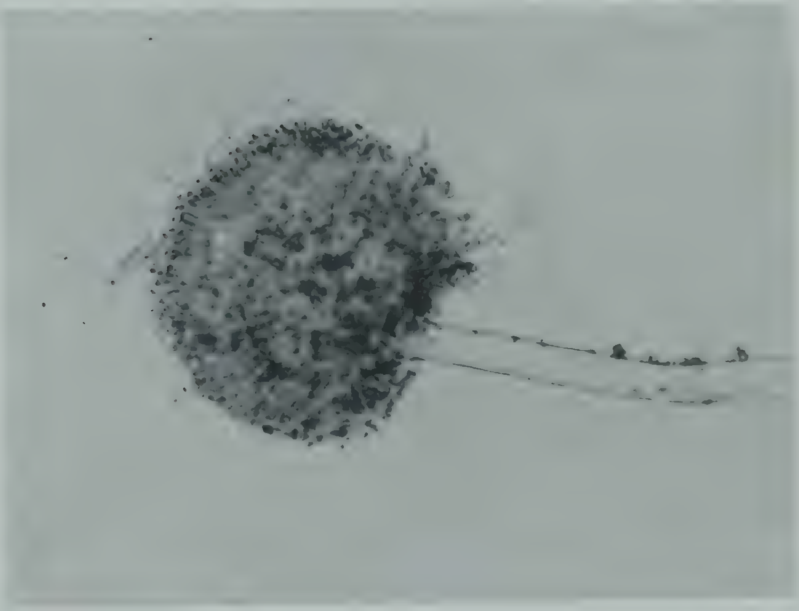


Fig. 100.—*A. niger*—head as seen after bleaching. × 250.

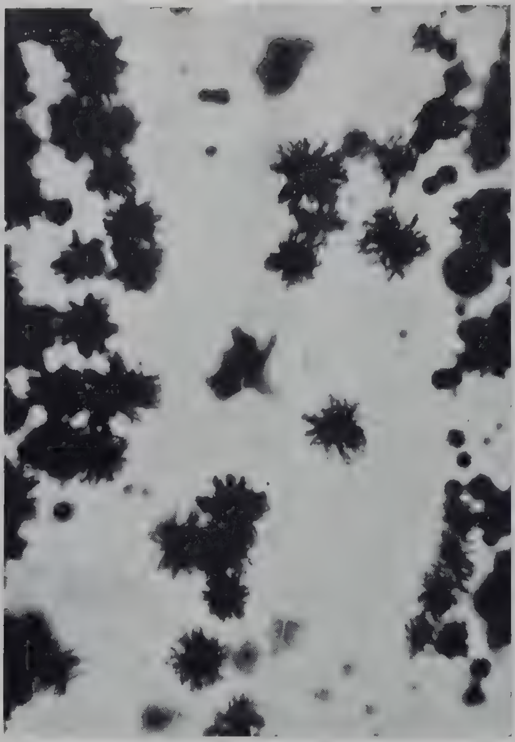


Fig. 101.—*A. Wentii*—mature heads as seen in Petri dish. × 20.

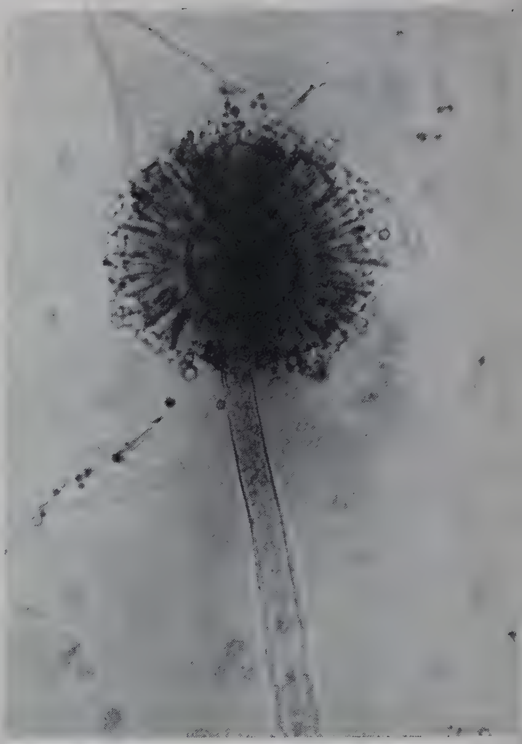


Fig. 103.—*A. Wentii*—typical head. × 250.

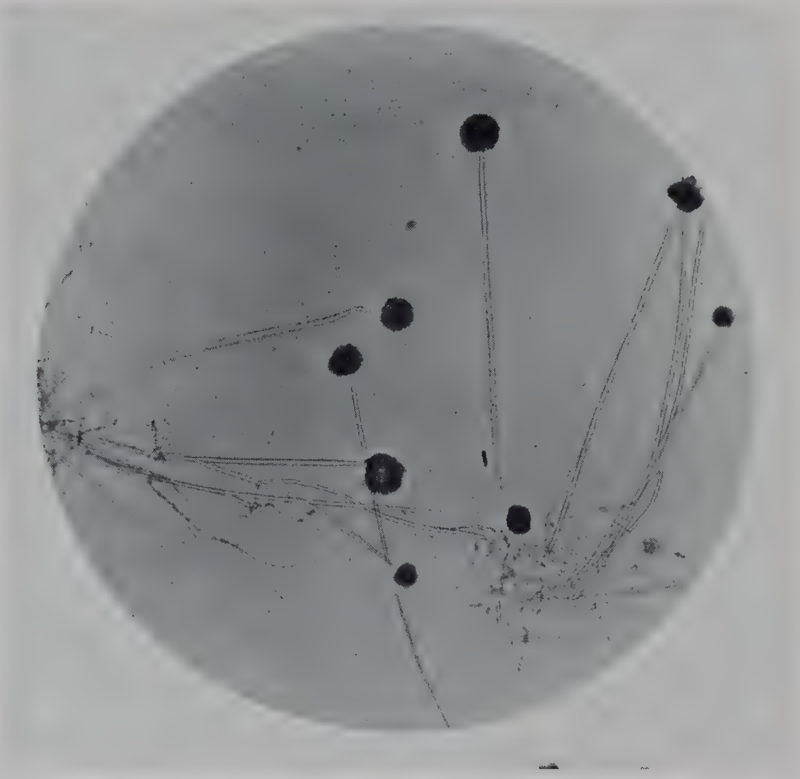


Fig. 102.—*A. Wentii*—portion of young culture (mounted). × 25.

a mutant of a very different type. Development is normal up to the production of secondary sterigmata. Then, instead of unbranched chains of black spores being produced, branched outgrowths are formed from the sterigmata, these sometimes producing single terminal conidia. Mature colonies are grey to greenish black.

As indicated earlier, species belonging in this group have interesting biochemical characteristics, and workers who are concerned with this side of their activities will need to separate strains on biochemical, rather than on morphological grounds.

A. Wentii Wehmer. This species is well marked and easy to recognize. The aerial mycelium forms dense floccose masses, white or tinged with pink or rose, completely filling culture tubes when the food supply is abundant. On some natural substrata it is found as a dense, furry, polychromatic growth. The conspicuous spore heads are coffee-coloured, up to 500μ or even 800μ in diameter, very ragged-looking when mature, and borne on long slender stalks. The stalks are smooth and colourless; sterigmata in two series with the primaries varying very much in length; and the conidia globose or nearly so, about 5μ in diameter, roughened or pitted. Fig. 101 shows mature heads in a living culture, Fig. 102 two small portions picked off from a young colony and showing the long smooth stalks, Fig. 103 a specimen mounted in lactophenol.

A. ochraceus Wilhelm. The name covers a series of strains which are of common occurrence and wide distribution. Colonies are ochre-coloured or buff. Spore heads are large and conspicuous, at first globose, then splitting into two or more columnar masses, frequently of a shape and colour recalling sheaves of corn (Figs. 104, 105). The stalks are long, usually 10μ or more in diameter, yellow, and pitted so as to appear definitely rough (see Fig. 106); vesicles globose; sterigmata always in two series; conidia globose or nearly so, smooth or delicately roughened, about 2·5μ in diameter, occasionally larger. Some strains form abundant heads and no sclerotia; others produce masses of orange to brown sclerotia and comparatively few heads.

A. sulphureus (Fresenius) Thom and Church. A number of strains, which are conveniently grouped as *A. sulphureus*, resemble *A. ochraceus* in general aspect and dimensions but differ in that the conidial colour is clear yellow instead of buff. As in *A. ochraceus*, some strains produce abundant sclerotia.

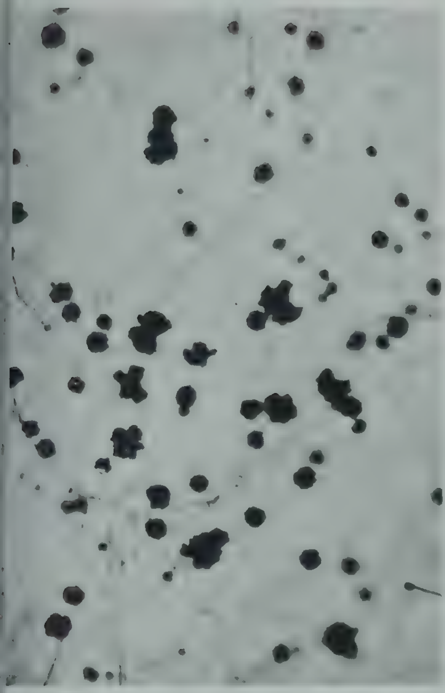
A. tamarii Kita. The colony colour in mature cultures is brown to deep brown or reddish brown, but young cultures frequently show a distinct greenish tone and have the peculiarly lush appearance of a typical *A. flavus*, indicating a very near relationship to the latter species. The heads are large, at first globose, but becoming loosely radiate, with single chains of spores visible under low magnification. Vesicles are thin-wallled and very easily crushed when mounting; sterigmata are typically in two series, but some heads bear one series and it is not unusual to find single and branched sterigmata in the same head; conidia are globose to pyriform, 5–7·5μ in diameter, with nodules of brown colouring matter between the two walls and hence appearing coarsely roughened except under high magnification. *A. tamarii* resembles *A. flavus-oryzæ* in producing kojic acid (see below).

The A. flavus-oryzæ Group. Two species are frequently mentioned in the literature, *A. flavus* Link and *A. oryzæ* (Ahlburg) Cohn, the former yellowish green to true green, and the latter more distinctly yellow and becoming yellowish brown. Examination of numerous strains has shown that there is no sharp line of distinction, and all such races are now usually lumped together as *A. flavus-oryzæ.*

Colonies grow rapidly sometimes definitely floccose,, sometimes consisting minaly of closely crowded, erect conidiopores. Stalks are long, mostly 8–15μ in diameter, and usually so densely pitted as to appear spiny. Heads vary much in size and bear one or two series of sterigmata as in *A. tamarii.* Spores are globose, mostly 3·5–5μ in diameter, occasionally larger, and very rough. Brown sclerotia are known in some strains.

A series of strains of somewhat different appearance from the rest of the group are represented by *A. effusus* Tiraboschi. Young cultures are deeply floccose, white, then dirty greenish yellow, with reverse yellow to rose. Old cultures are dirty brown and have a distinctive cottony appearance. The stalks arise from the aerial mycelium, and it is not unusual to find fertile hyphæ consisting of almost uninterrupted series of foot-cells. The heads otherwise are as in *A. flavus-oryzæ* (Fig. 107).

One of the most interesting features of this group of *Aspergilli* is their power of producing a substance known as "kojic acid." This substance was first obtained by Saito in 1907, from the powdered mycelium of *A. oryzæ.* It was later investigated by Yabuta, who showed that it could be obtained by growing the mould on a synthetic medium with glucose as the sole source of carbon, and eventually proved it to be 5-hydroxy-2-hydroxymethyl-γ-pyrone (Yabuta,



. 104.—*A. ochraceus*—heads as seen in Petri dish. × 20.

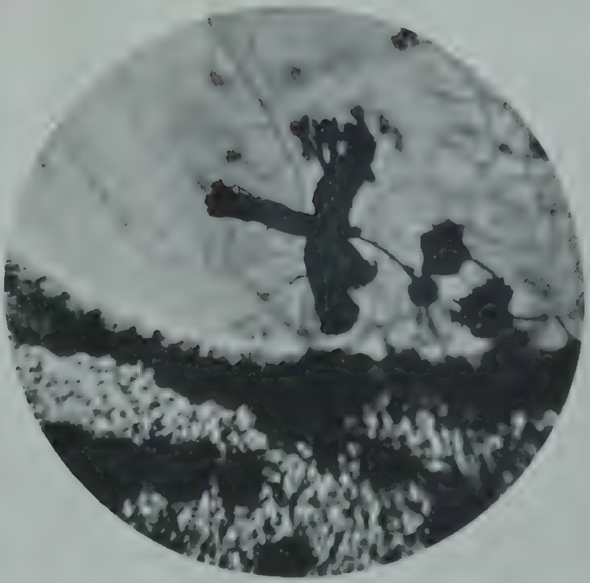


Fig. 105.—*A. ochraceus*—single large head in old culture, showing characteristic splitting. × 25.

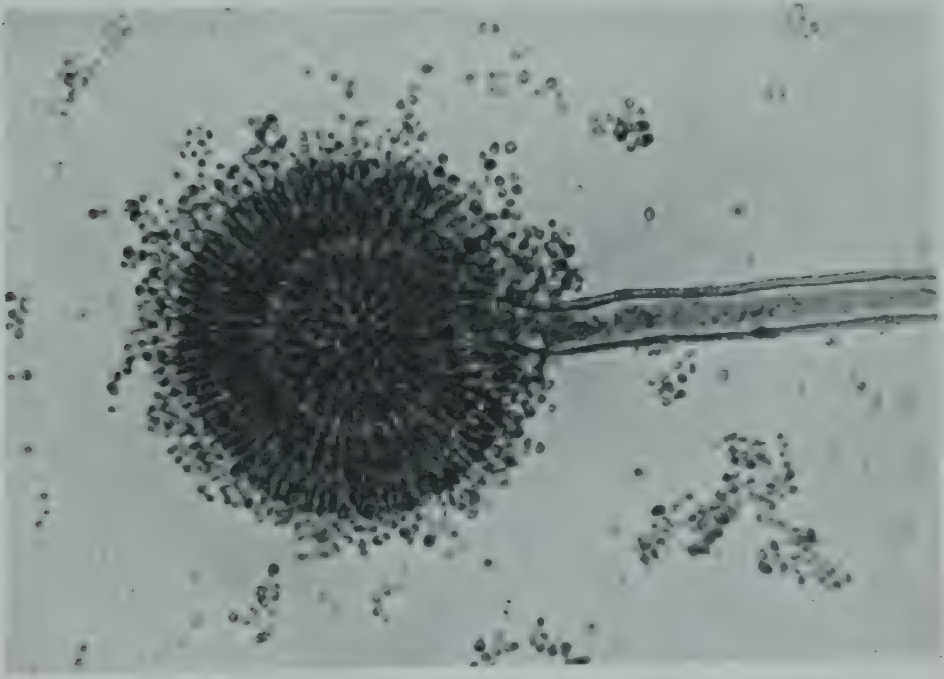


Fig. 106.—*A. ochraceus*—typical head. × 500.

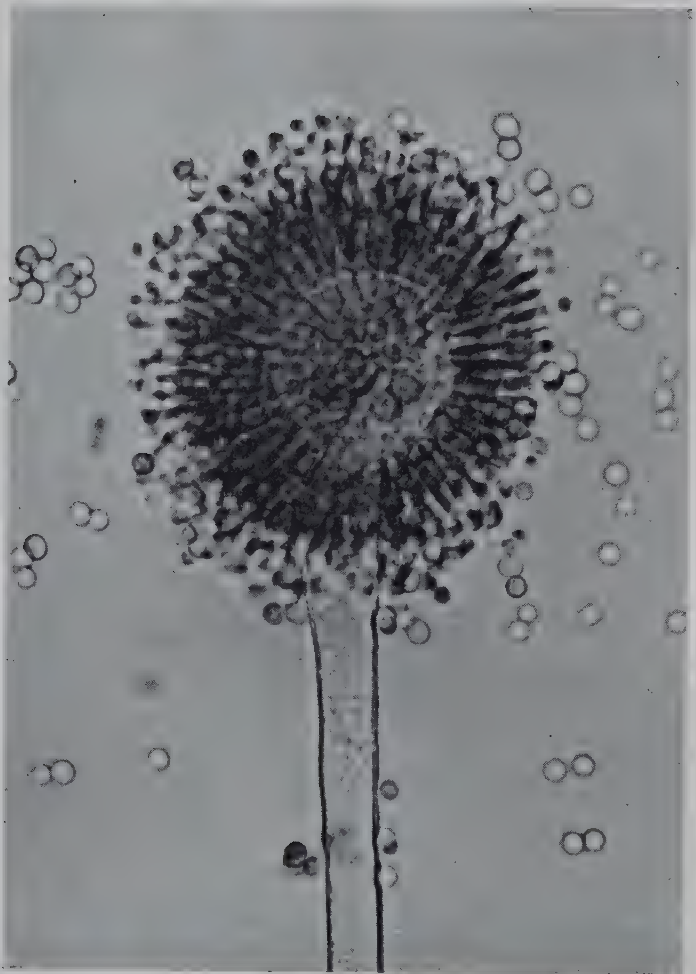


FIG. 107.—*A. effusus*—the type of head commonly seen in slides of any strain of the *A. flavus-oryzæ* series.

1924). Raistrick and co-workers independently discovered kojic acid in 1923, and their work is of great interest in showing that a number of *Aspergilli*, when grown on Czapek's solution, produce kojic acid, and that *all* of these belong in the series represented by *A. flavus*, *A. oryzæ* and *A. tamarii* (Birkinshaw *et al.*, 1931). Kojic acid, in water solution, gives a very intense and characteristic blood-red colour with ferric chloride, and this reaction forms a useful diagnostic test for strains in this group. The simplest method of conducting the test is to grow the mould on the liquid medium for any length of time between two and four weeks, filter the solution and add a few drops of a 10 per cent solution of ferric chloride. Raistrick and co-workers grew all their strains on Czapek's solution, but found that the intensity of the reaction varied and that one culture of *A. flavus* gave no reaction. In the author's experience the test is more certain, and the reaction more intense and uniform, if the Raulin-Thom medium is used. *All* strains so far examined give a strongly positive reaction under these conditions. Reference is made in Chapter XIV to the industrial uses of *A. flavus-oryzæ*.

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CHAPTER IX

PENICILLIUM AND RELATED GENERA

THE *Penicillia* are closely related to the *Aspergilli* and are just as widespread and omnivorous. Like the *Aspergilli*, many of them are common and serious agents of destruction, but, on the other hand, there are several species which are best known as the means of conducting industrial fermentations. Probably the most important of these are the cheese moulds, the *P. roqueforti* and *P. camemberti* groups. Thom's studies of the occurrence and mode of action of these moulds has made it possible for production of cheeses of the Gorgonzola, Stilton, Roquefort and Camembert types to be carried on anywhere with success, instead of being confined in special regions. The first attempts to produce citric acid commercially by mould fermentation used *Penicillia*, of the group formerly known as *Citromyces*, although modern citric acid manufacture is carried on with the aid of *Aspergillus niger*, owing to the better yields obtained. Comparatively recently a species of *Penicillium* has been used for the large-scale production of gluconic acid. The studies of Raistrick and his school have shown that the *Penicillia* show the most varied metabolic activity, different species being capable of synthesizing from glucose a bewildering variety of substances of complex chemical constitution, and it is conceivable that such work will lay the foundations of future commercial processes employing moulds. Amongst many interesting results already obtained may be mentioned the production of ergosterol, the parent substance of Vitamin D, and of a number of substances closely related to ascorbic acid (Vitamin C).

Unfortunately the taxonomy of the genus presents much greater difficulty than that of the related genus, *Aspergillus*. There is not the same wide range of conidial colour to serve as a convenient basis of classification, most *Penicillia* being some shade of green during the growing period, and, in addition, the colours are not usually stable but change with varying cultural conditions and with the age of cultures. Further, the number of species of *Penicillium* greatly exceeds the number of *Aspergilli*. The number of specific names which have been bestowed runs into many hundreds and, whilst many of these are synonyms or probable synonyms, there are still about 150 which, in the present state of our knowledge, seem to represent good species.

In view of the difficulties of classification it is not surprising that *Penicillium* has been the subject of several monographs and extensive papers, by Dierckx (1901), Thom (1910), Westling (1911), Sopp (1912), Thom (1915), Biourge (1923), Zaleski (1927) and Thom (1930), to mention only the most important. Thom's latest classification (1930) is the one generally used at the present time, although in some ways it is imperfect and difficult to use. The following is a diagnosis of the genus, embodying Thom's conceptions and introducing several terms which are commonly used in descriptions of species.

Penicillium Link. Mycelium hyaline or pale or brightly coloured, septate, either predominantly submerged or partly submerged and partly aerial, with aerial portion either loose to definitely floccose or partially as ropes of hyphæ; fertile branches (conidiophores) arising from and more or less perpendicular to submerged or aerial hyphæ, either simple or to some degree aggregated into fascicles or compacted into definite coremia, septate, smooth or roughened, terminating in a broom-like whorl of branches (the *penicillus*), consisting of a single whorl of spore-bearing organs (sterigmata) or twice to several times verticillately branched, with the branching system symmetrical or asymmetrical, the final branches being the sterigmata; conidia produced as in *Aspergillus*, by abscission, forming unbranched chains, globose ovoid or pyriform, smooth or rough, in most cases green during the growing period; perithecia known in a few species, either sclerotium-like or thin-walled; sclerotia formed by several species and of some diagnostic importance.

Two other terms require explanation. In penicilli with more than one stage of branching the branches bearing the sterigmata are called *metulæ* and the branches supporting the metulæ are known as *rami* (Fig. 108).

Langeron (1922) proposed the generic name *Carpenteles* for a species, described by Brefeld as *P. glaucum*, which produces sclerotia which slowly ripen to become thick-walled perithecia, and Shear (1934) has recently revived the name to cover Brefeld's species and a few others discovered within the last few years. Until such time as we have a new monograph on the genus *Penicillium* the question of nomenclature for the ascosporic species may be left *sub judice*.

Thom (1930) recognizes three other genera, closely related to *Penicillium* and including a few very common and important species.

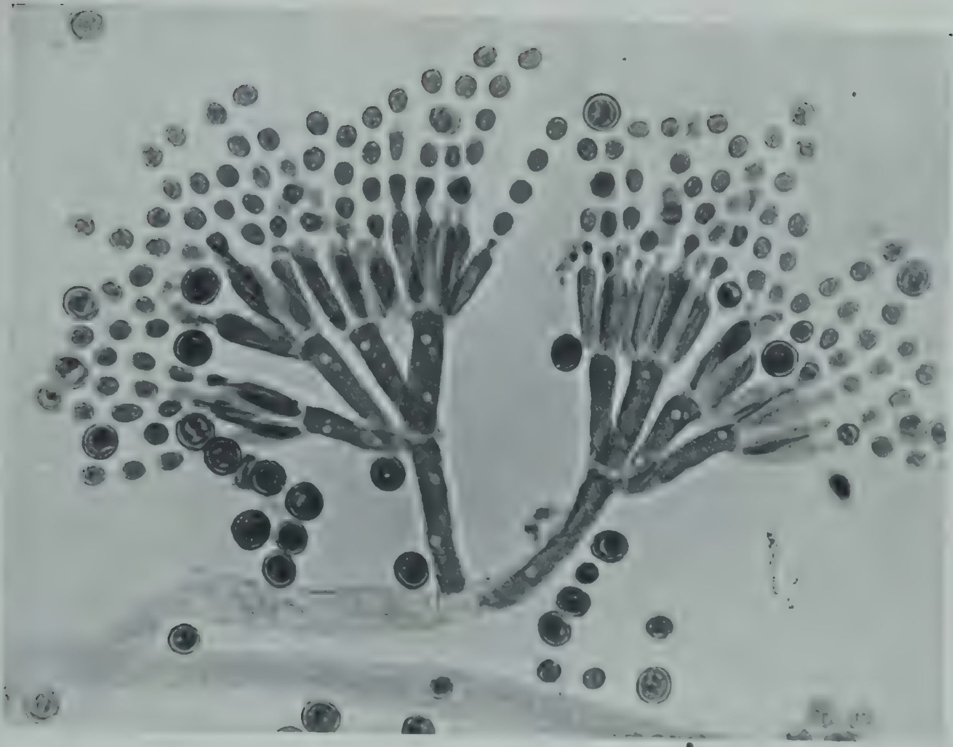


FIG. 108.—*Penicillium*—a typical "penicillus" with several stages of branching and showing the mode of formation of the conidia. × 1000.

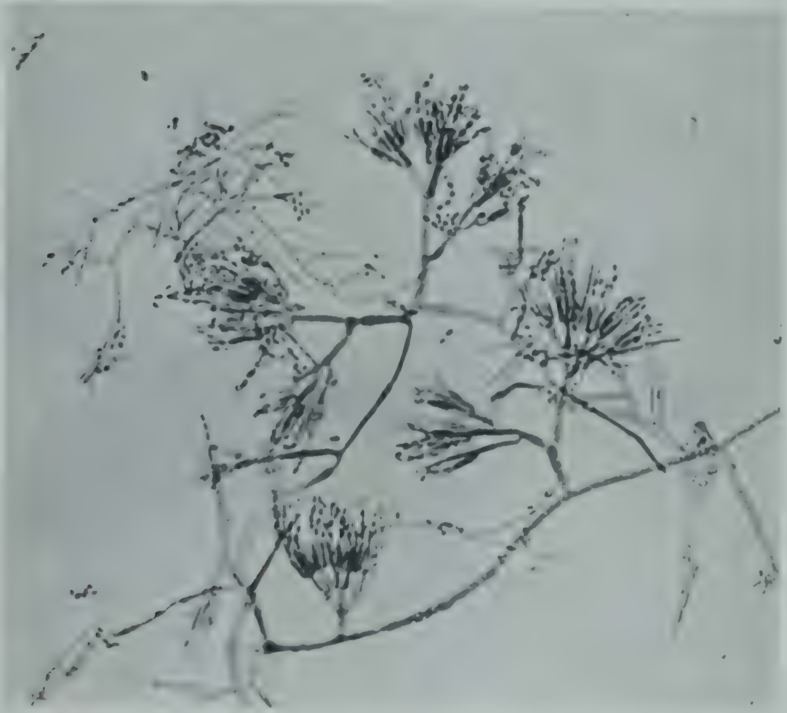


FIG. 109.—*Gliocladium roseum*—conidiophores. × 250.

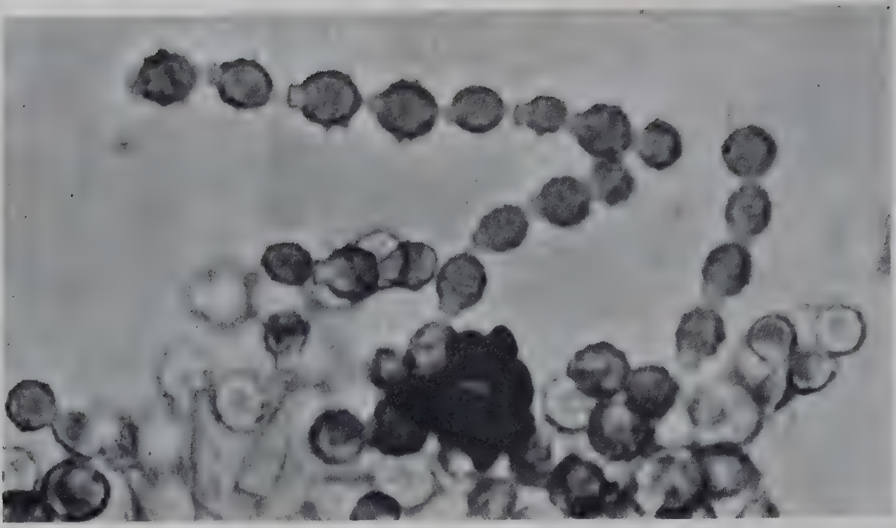


Fig. 110.—*Scopulariopsis brevicaulis*—spores, some showing the typical thickened ring. × 1000.

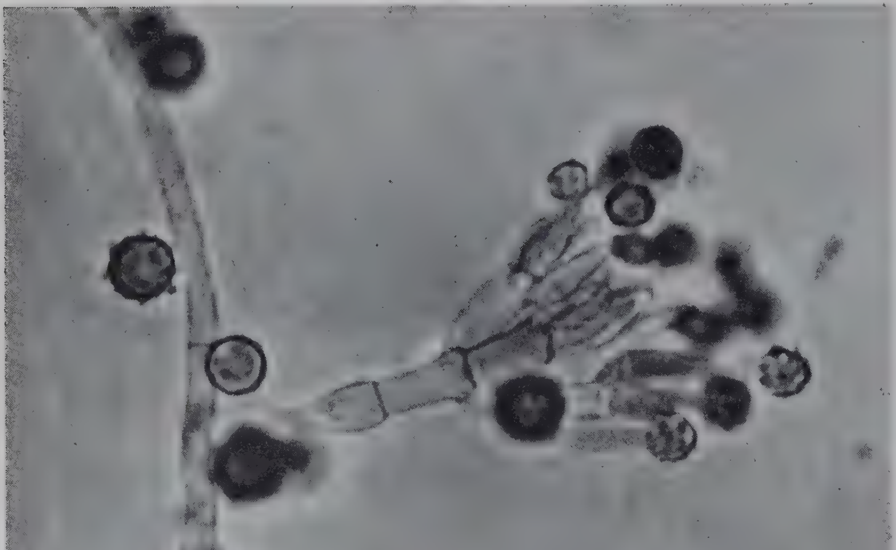


Fig. 111.—*Scopulariopsis brevicaulis*—penicillus. × 1000.

Gliocladium differs from the true *Penicillia* in that a mucilaginous substance is produced by the fruiting organs, and the conidia, instead of standing in detached chains, adhere together or, in the typical species, lose entirely the catenary formation and form solid, slimy balls. The line of separation is not a sharp one, but there are a number of strains in which the distinctive character is well marked and unmistakable.

G. roseum (Link) Bainier is the typical member of a series forming salmon-pink to rose-pink colonies, loosely floccose in texture, with conidia 5–7µ by 3–5µ, forming true slime balls somewhat tardily.

G. deliquescens Sopp forms at first *Penicillium*-like colonies of a clear yellow green colour later turning darker and becoming slimy, with conidia mostly 3–3·5µ by 2–2·5µ.

G. catenulatum Gilman and Abbott grows as floccose to funiculose colonies, white at first, becoming clear dark green in scattered patches, with conidial chains in long columns, held together by slime, and smooth conidia, 4–7·5µ by 3–4µ.

In all the species the penicilli are mostly several times verticillate, with the branches becoming successively more slender (Fig. 109).

Scopulariopsis. The conidial colour is never green as in most of the true *Penicillia*. The penicillus is irregular in form, varying from complex branching systems to single sterigmata produced on short branches from the aerial mycelium. The most distinctive feature is the conidium, which is usually more or less lemon-shaped, pointed at one end and at the other having a thickened ring with a central pore (Figs. 110, 111).

S. brevicaulis Bainier is of common occurrence on all kinds of material. It forms brownish, cottony colonies of somewhat thin texture. The conidia are large, 6–7µ in long axis, lemon-shaped, showing well-defined ring and pore and with the outer wall coarsely roughened. A variety, *S. brevicaulis var. alba* Thom, is similar except that the colony colour is white and the spores are somewhat larger. A second variety, *S. brevicaulis var. glabra* Thom, differs from the parent species in that the spores are smooth; it also forms black sclerotia. A special point of interest is that all species of this genus (and of *Pœcilomyces* too) can liberate arsenic in the form of very poisonous gaseous compounds from any substrate containing even a trace only of this element. When grown on ordinary gelatine, for example, the garlic-like odour common to arsine and its alkyl derivatives is distinctly noticeable. There have been one or two serious cases of arsenic poisoning due to the growth of *S. brevicaulis* on wall-papers coloured with Paris green, and it has been proposed to use this species for detecting minute traces of arsenic in suspected materials, instead of applying the usual chemical methods.

Pœcilomyces Colony colour varies from white to snuff brown, and the texture is loose cottony or ropy. The penicillus varies much in complexity (much as in *Scopulariopsis*) and the ovate conidia are produced in very long tangled chains. The sterigmata are of very characteristic shape, broad at the base, then tapering sharply to long, slender tips which are usually bent away from the main axis (Figs. 112, 113). Most strains also produce so-called "macrospores," which are comparatively large, globose to ovate spores, borne singly or in clusters, and usually found on the mycelium close to the substrate or in the submerged mycelium (Fig. 114).

P. varioti Bainier is of widespread occurrence and grows well on almost any kind of substrate. Colonies are pale, dull brown in colour, loosely floccose or funiculose, with spores approximately 6μ by 2·5–3μ. It has often been identified as *Spicaria elegans* Harz and there is still some doubt as to whether the latter is a separate species.

The Penicillia proper

The multitude of species fall naturally into three main divisions, recognized as a primary basis of separation by all modern authorities. The Monoverticillata (to use Thom's term), equivalent to the section Aspergilloides of several other authors and to Wehmer's genus *Citromyces*, have penicilli consisting of a single whorl of sterigmata (Fig. 115). The Biverticillata-Symmetrica have a compact whorl of metulæ, each bearing sterigmata, the whole penicillus being approximately symmetrical about the axis. In addition, the spores are always ovate or pyriform and are borne on slender, tapering (acuminate) sterigmata (Fig. 116). The third and largest group, the Asymmetrica, includes all species in which the penicillus is branched more than once and is asymmetrical, or, if approximately symmetrical, has not the compacted structure and the slender, acuminate sterigmata of the Biverticillata-Symmetrica.

Each large group is sub-divided according to colony characteristics whether velvety, lanose, funiculose or fasciculate, and it is essentia that Thom's conceptions of these terms should be thoroughly under stood if his keys are to be used for identifications. The velvet colony has the aerial growth consisting chiefly of conidiophores arising from submerged hyphæ and giving exactly the effect of a

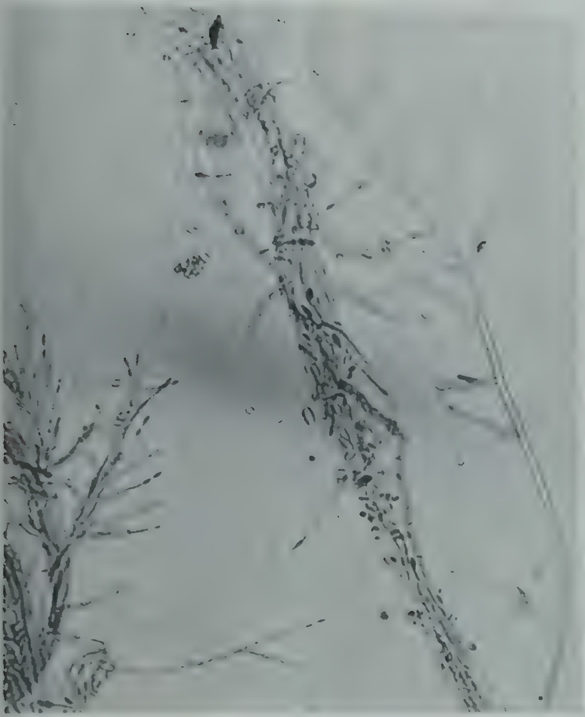


FIG. 112.—*Pœcilomyces varioti*—ropes of hyphæ and various types of spore-bearing structures. × 250.

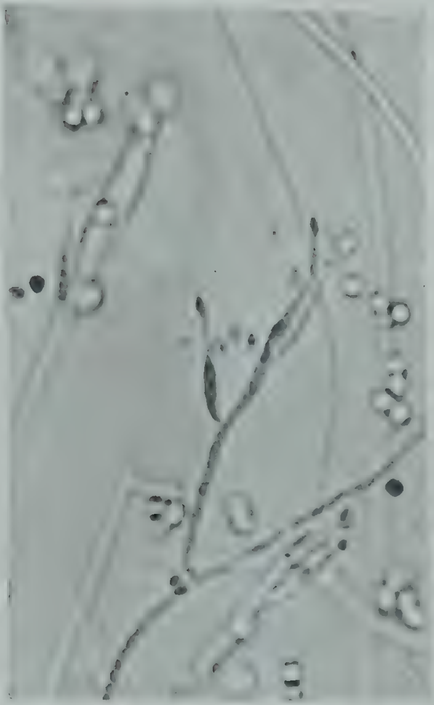


FIG. 113.—*Pœcilomyces sp.*—characteristic sterigmata. × 800.

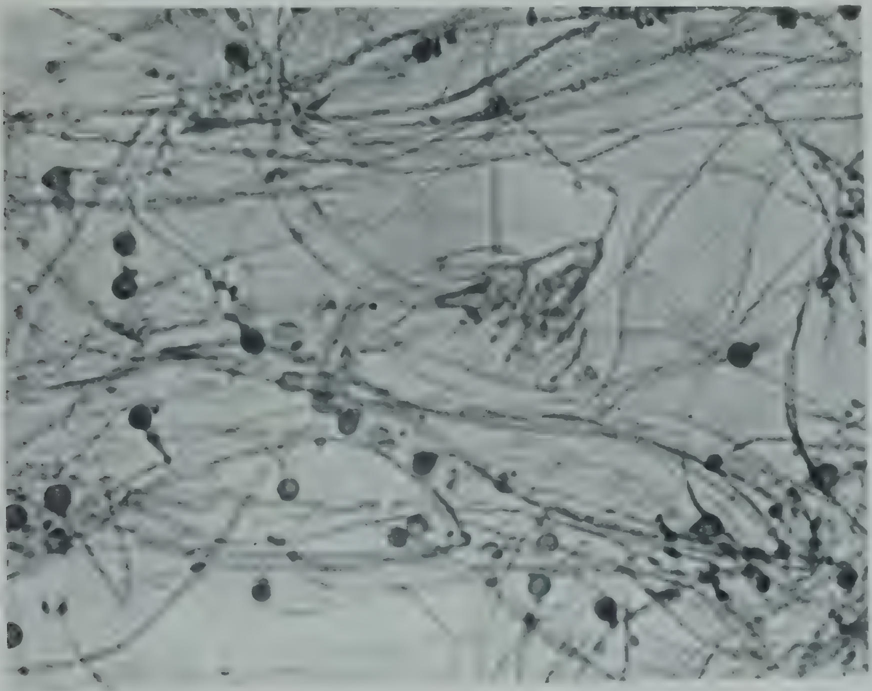


FIG. 114.—*Pœcilomyces sp.*—macrospores. × 500.

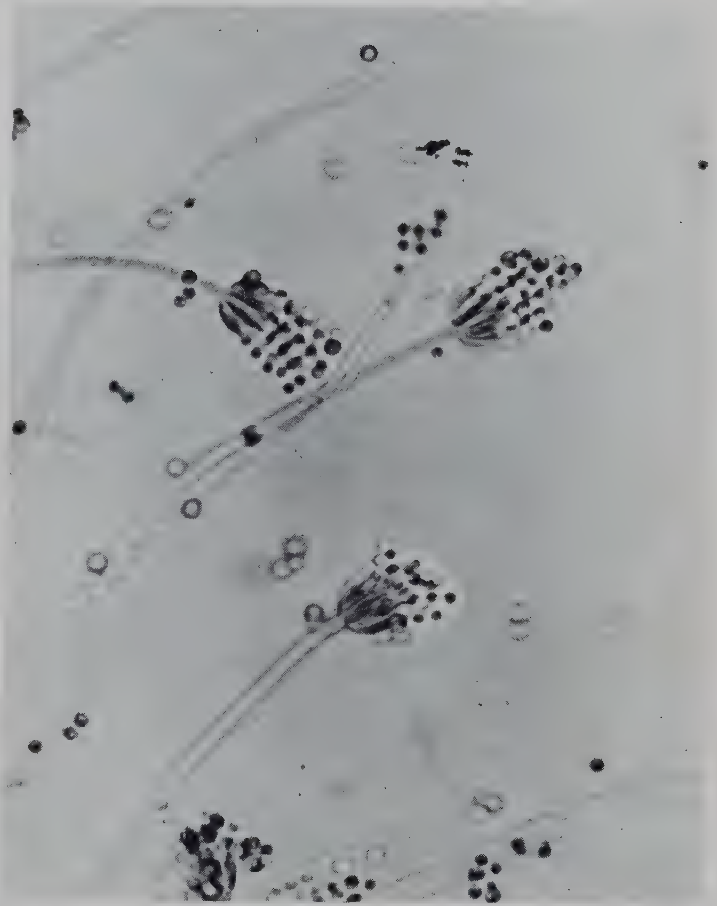


FIG. 115.—*Penicillium spinulosum*—typical monoverticillate conidiophores. × 500.

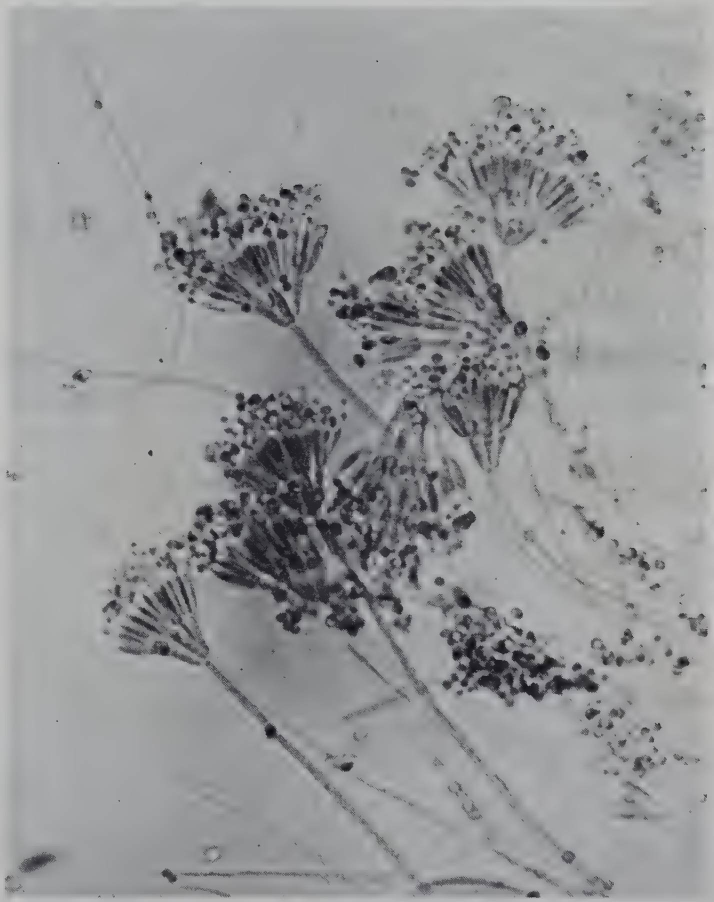


FIG. 116.—*P. luteum*—symmetrically biverticillate conidiophores. × 500.

short pile velvet. In lanose species there is a tangled mass of aerial mycelium and the conidiophores arise mainly from these aerial hyphæ, the points of origin being quite away from the substratum. In the Funiculosa part or most of the aerial hyphæ are aggregated into trailing ropes, a character which is usually easy to recognize under a low power of the microscope or even with a good hand lens. Species belonging to the fasciculate sections produce conidiophores, mostly arising from the substratum, which are not evenly distributed, as in the velvety type of colony, but are more or less in clusters, giving a granular or mealy appearance to the colony, or compacted into definite coremia. Thom recommends that colony habit should be studied by cutting thin radial sections from colonies grown in Petri dishes and examining the cut edges of the sections under the microscope. It is, however, usually possible to come to a decision by examination of the edges of the sterile areas which occur between colonies when several colonies are grown in the same dish. Probably the most difficult species to place are those in which fasciculation is slight and whose microscopic appearance is little different from that of the velvety species. In such cases it is often a help if the mould is grown as a single colony on a very thin layer of medium in a Petri dish. Under these conditions some strains of *P. expansum*, for example, will produce concentric rings of small, but definite, coremia when, on slopes, they give colonies which are almost truly velvety.

One of the great difficulties in attempting to identify *Penicillia* is that most of the cosmopolitan species appear to be to some extent unstable. Some species tend to vary in successive cultures made, in the laboratory, under apparently identical conditions, but these, with few exceptions, are approximately stable over periods long enough to permit of identification and the instability in question is something more fundamental. With the more common types of organism it is frequently impossible to find an exact fit for a strain in any published descriptions of species, although the strain in question is readily placed as "near to" two, or perhaps three, species previously described. Biourge (1923) gave specific names to all the members of such related series examined by him, but Thom has shown that, when hundreds of strains are examined, differences become less perceptible, and the only alternatives are the bestowal of new specific names for almost every strain isolated, and the more practical one of lumping together nearly related strains as a group species. Most living things show, under natural conditions, some tendency to saltation and it is perhaps reasonable to expect that a mould of common and widespread occurrence should be found as a series of slightly different strains, even when such tendency to saltate is not marked. In all the main sections of *Penicillium* there are these group species and, except for the specialist, it is unnecessary to attempt to carry identifications further than the fitting of a particular strain into its group.

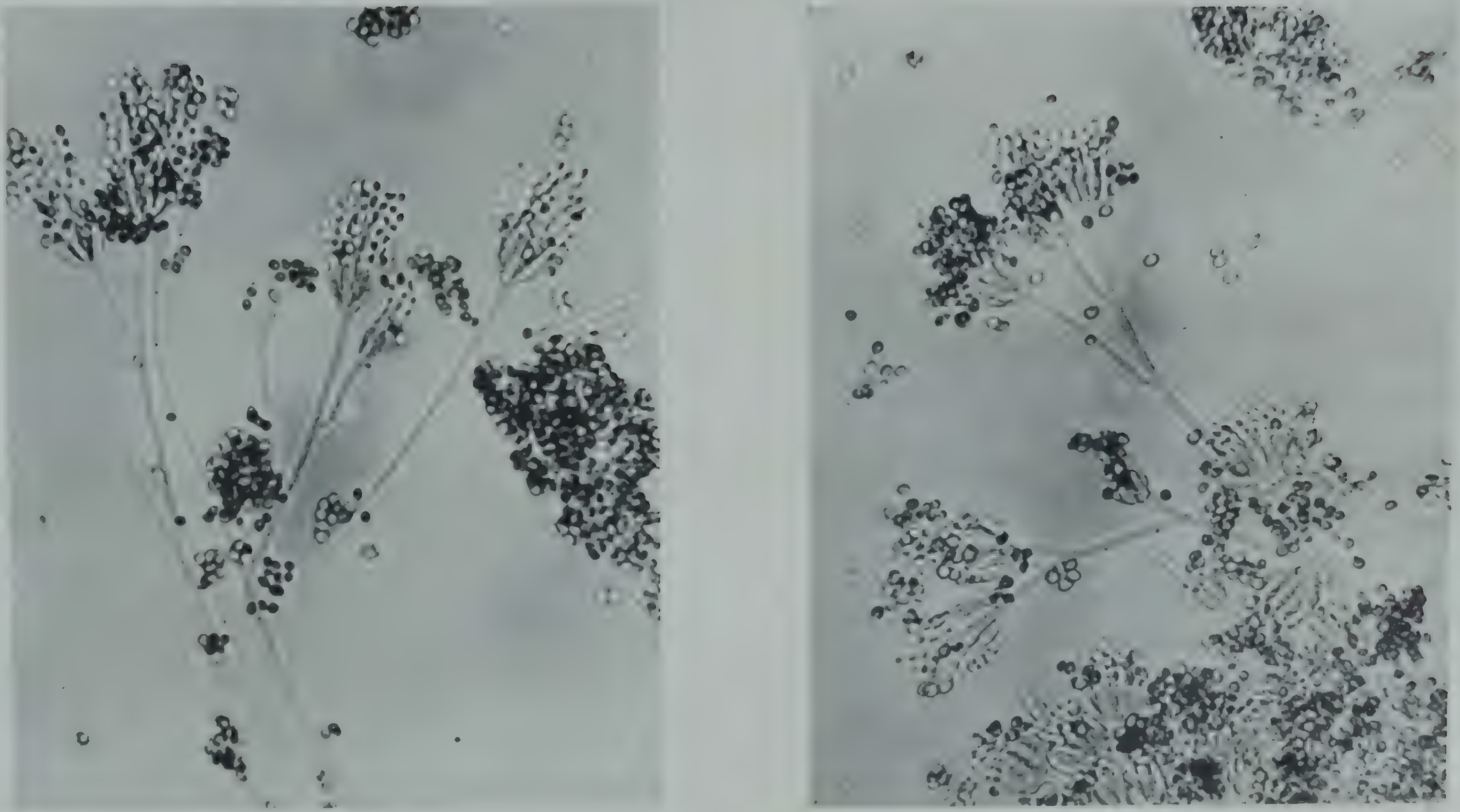
In view of the large number of known species it is impossible to describe here more than the best known and common types. The new-comer to a study of this genus is strongly advised to familiarize himself with a number of such forms (by examination of type species, cultures of which can be purchased from any of the well-known culture collections) before attempting to identify unknown strains. This is the most rapid way of acquiring precise conceptions of the diagnostic terms used to define groups and species.

The Monoverticillata

Thom devides these into two series, Monoverticillata-Stricta and Monoverticillata-Ramigena. In the first the majority of the conidiophores are simple and unbranched or, at most, sparingly branched at some distance from the tip. In the Ramigena the conidiophores are mostly branched, but in such a way that each monoverticillate penicillus retains its individual character. Species of this series are few in number compared with the Stricta and are not of common occurrence. Actually there is no great difference between species of this series and those of the series Lanata-Divaricata (p. 150), and those wishing to identify species having this type of penicillus should work through both series. The illustrations of *P. Charlesii* (Figs. 117, 118) will give an idea of the typical structure which defines the section, and will assist in placing any strains which may be encountered.

The Monoverticillata-Stricta are divided into four groups according to colony texture, Sclerotigena, Floccosa, Funiculosa and Velutina, names which are self-explanatory. Many species have been described, but a large number of them may be aggregated under a few specific names, which serve to place the commonly occurring forms near enough for most purposes.

P. Thomii Maire is readily distinguished by its abundant production, particularly in freshly isolated cultures, of pinkish sclerotia. It has been reported as parasitic on Orchid leaves (Moore, 1941).



FIGS. 117, 118.—*P. Charlesii*—variously branched conidiophores characteristic of the Monoverticillata-Ramigena. × 500.

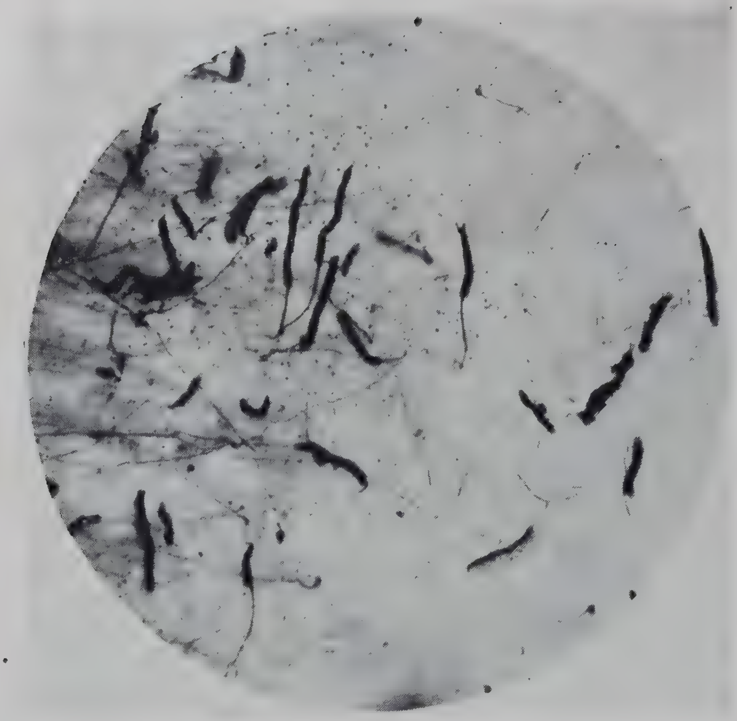


FIG. 119.—*P. frequentans*—columns of spore chains as seen in Petri dish. × 50.

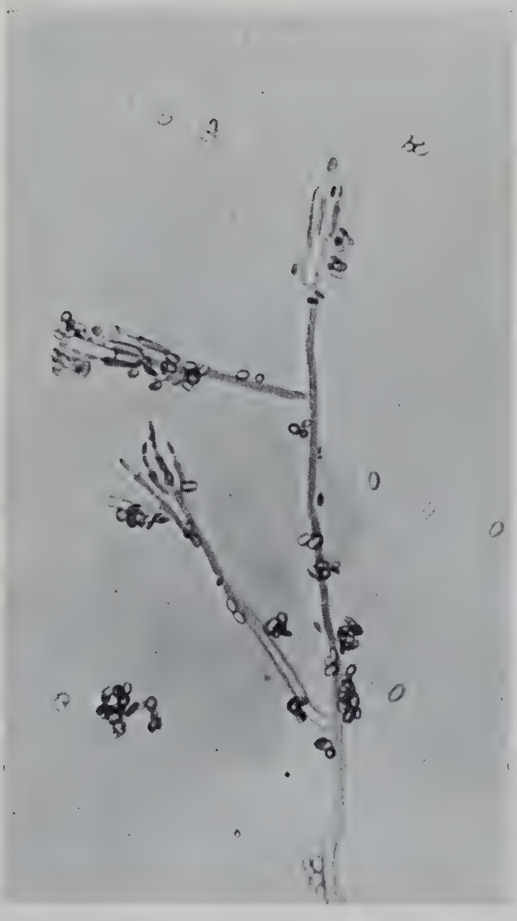


FIG. 120.—*P. digitatum*—conidiophores. × 250.

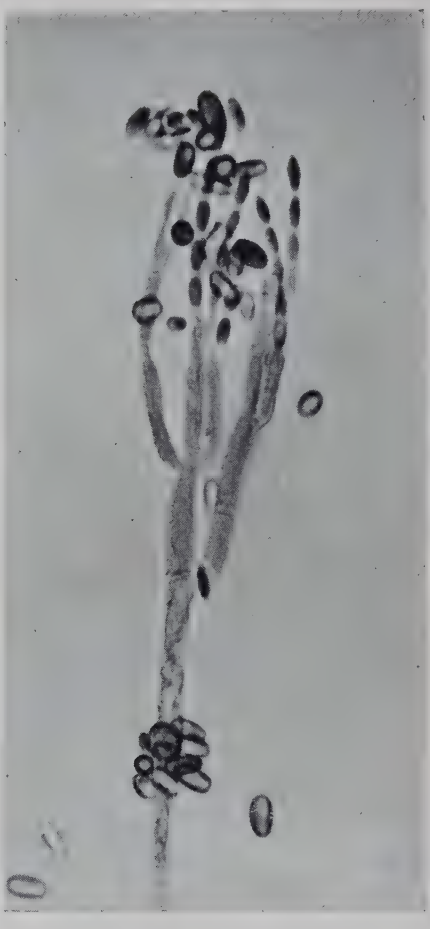


FIG. 121.—*P. digitatum*—conidiophore. × 500.

P. spinulosum Thom is the type species of the Stricta-Floccosa. Colonies are broadly spreading, and usually sparsely floccose, the amount of floccosity varying much in different strains. Colony colour is variously yellow green, blue green or grey green, becoming definitely greyish in old cultures, with broad white edge during the growing period; reverse of colony is normally white to cream but is pink or purplish in some strains. Conidia are produced in parallel chains usually more or less compacted into columns and are nearly globose, 3–4μ in diameter and delicately roughened.

This species is of common occurrence and has been found on such diverse materials as hair-cream, fibre-board, cotton yarns and leather, also as a common contaminant of Petri dishes in the laboratory. Certain strains differ from the normal in producing an intense purple colour in certain culture media. On solid media the appearance is very distinctive, there being a central patch of green, then a ring of bright yellow and an outermost zone of purple. Birkinshaw and Raistrick (1931) have isolated the purple pigment and shown it to be a methoxydihydroxytoluquinone. A solution of the quinone is turned yellow on acidification and the yellow colour which develops in culture media is due to the gradual accumulation of citric acid. Such strains, when kept in artificial culture, invariably lose, after a time, the power of producing the pigment.

The Stricta-Funiculosa include a number of species which have been mostly described as occurring in soil. They do not appear to be of any importance in other connections and Thom's book should be consulted for details.

The Stricta-Velutina group includes two species of importance.

P. frequentans Westling, synonym *P. glabrum* (Wehmer) Westling, is an exceedingly common type of organism. Although individual strains show considerable variation, there is no difficulty in placing any particular strain in the group species. Growth on all media is very rapid, surface normally a rich green becoming darker and browner in age, with reverse yellow to deep brown. The tip of the conidiophore is swollen and bears a single whorl of sterigmata 9–10μ long. Conidial chains are packed into very long solid columns (Fig. 119), with conidia globose, mostly smooth, 2·6–3μ in diameter. When grown on Czapek's solution the liquid, after ten to fourteen days' incubation, gives an intense olive green on addition of ferric chloride, the reaction being due to the formation by the mould of a substance of complex constitution, named citromycetin (Hetherington and Raistrick, 1931*a*).

P. flavi-dorsum Biourge is typical of a series of closely related strains which appear to be fairly common in this country and which are important because of their abnormal resistance to most antiseptics. Colonies are pale bluish or greyish green with white margin and colourless reverse. Conidial chains are packed in long, solid columns, as in *P. frequentans*, with conidia 3–3·5μ in diameter, smooth or very slightly roughened.

The Asymmetrica

This is the largest of the three main divisions of *Penicillium*. It is sub-divided into six sections, Velutina, Brevi-compacta, Lanata-Typica, Lanata-Divaricata, Funiculosa and Fasciculata.

The ASYMMETRICA-VELUTINA are further sub-divided into six sections as follows:

Sect. 1. *Elliptica-Magna.* The chief species are *P. oxalicum* Currie and Thom, a soil organism, and *P. digitatum* Saccardo (Syn. *P. olivaceum* Wehmer). The latter occurs chiefly on oranges and other citrus fruits, producing a rot which spreads rapidly and is the cause of serious financial loss in the citrus fruit industry. It can readily be obtained in pure culture from mouldy oranges on which the infected areas are olive or yellowish green, not blue-green. It grows very poorly on Czapek agar but spreads rapidly on wort agar, producing smooth velvety colonies of a dull yellowish green colour. It is very difficult to prepare good slides showing typical conidiophores as the heads mostly fall to pieces when handled. When unbroken penicilli are found the aptness of the name is evident, for they have a distinct resemblance to a skeleton hand (Figs. 120, 121). All parts of the penicillus are large and the ovate spores, whilst varying much in size, are, on the average, bigger than those of any other *Penicillium*, being mostly 6–8μ by 4–7μ, but reaching 12μ or even more in long axis.

Sect. 2. *Divaricata.* The typical penicillus is biverticillate, but the metulæ tend to spread and each bears a group of short, blunt-pointed sterigmata, the whole disposed so that the penicillus appears as a bunch of monoverticillate heads (Figs. 122, 123). The spores are in a series of divergent solid columns, one column from each metula, giving a very characteristic appearance to colonies as seen under a low magnification (Fig. 124). The only important species is *P. citrinum* Thom, which is of world-wide distribution. Colonies are restricted in growth, velvety, dark bluish grey with a very narrow white edge and reverse varying from very pale to fairly deep

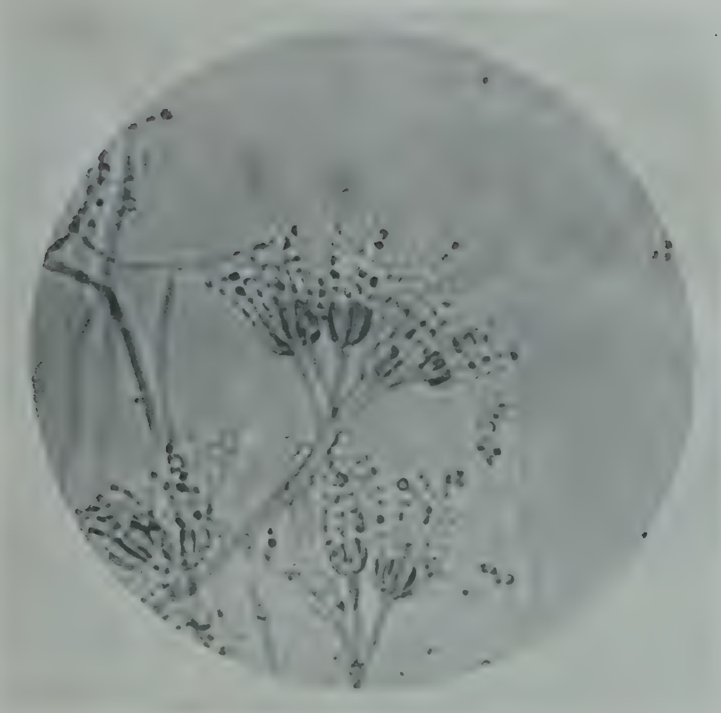


FIG. 122.—*P. citrinum*—divaricate penicillus. × 500.

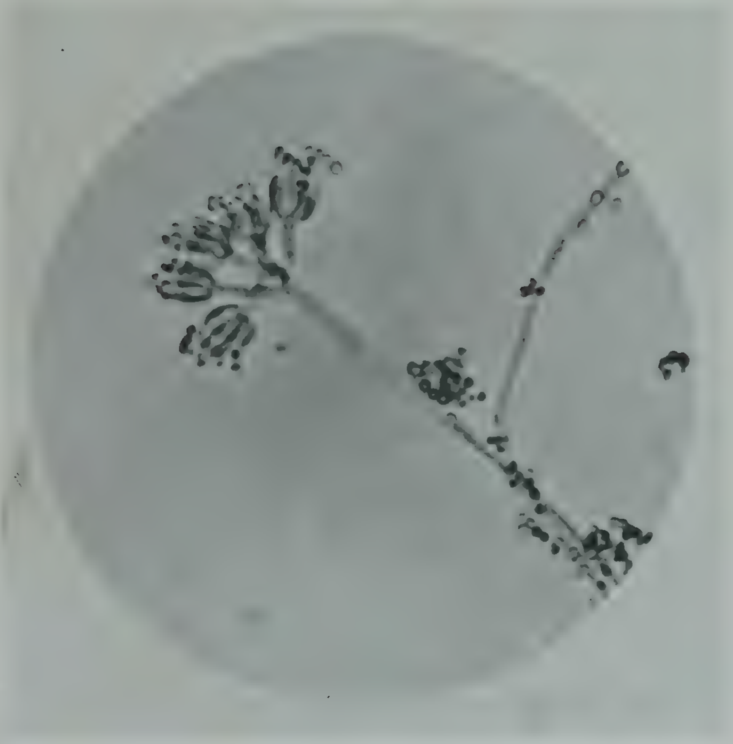


FIG. 123.—*P. Raistrickii*—divaricate penicillus. × 500.

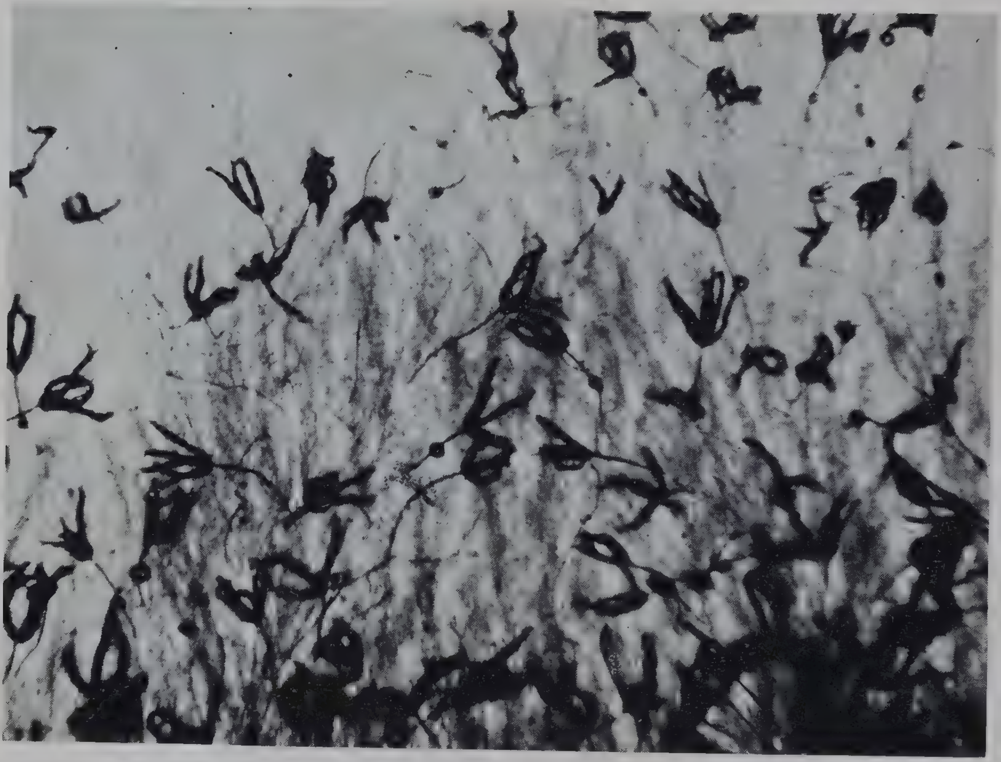


Fig. 124.—*P. Raistrickii*—divergent columns of spore chains as seen in Petri dish culture. × 100.

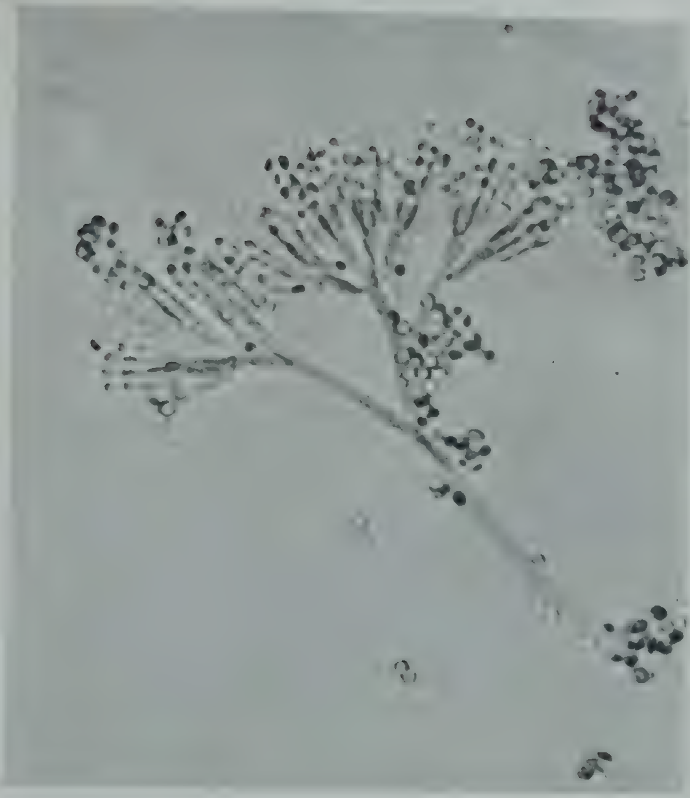


FIG. 125.—*P. chrysogenum*—penicillus. × 500.

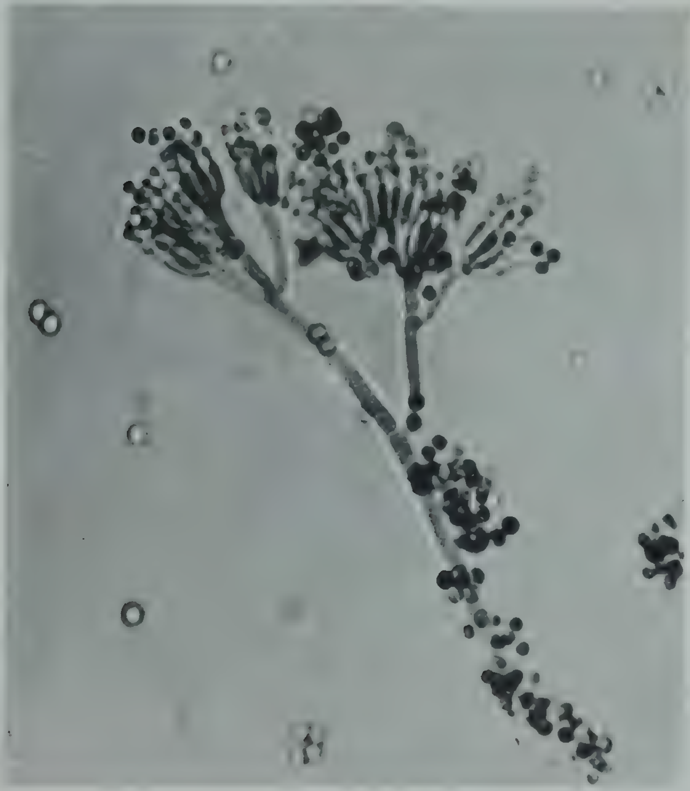


FIG. 126.—*P. roqueforti*—penicillus. × 500

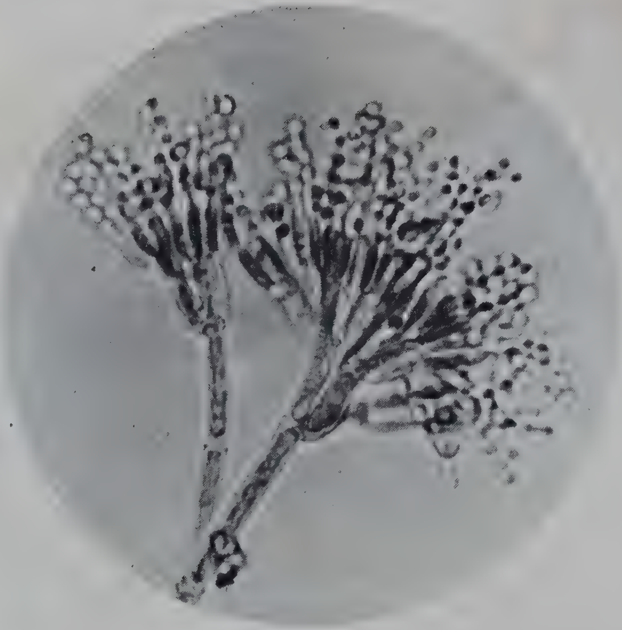


FIG. 127.—*P. brevi-compactum*—normal penicillus. × 600.

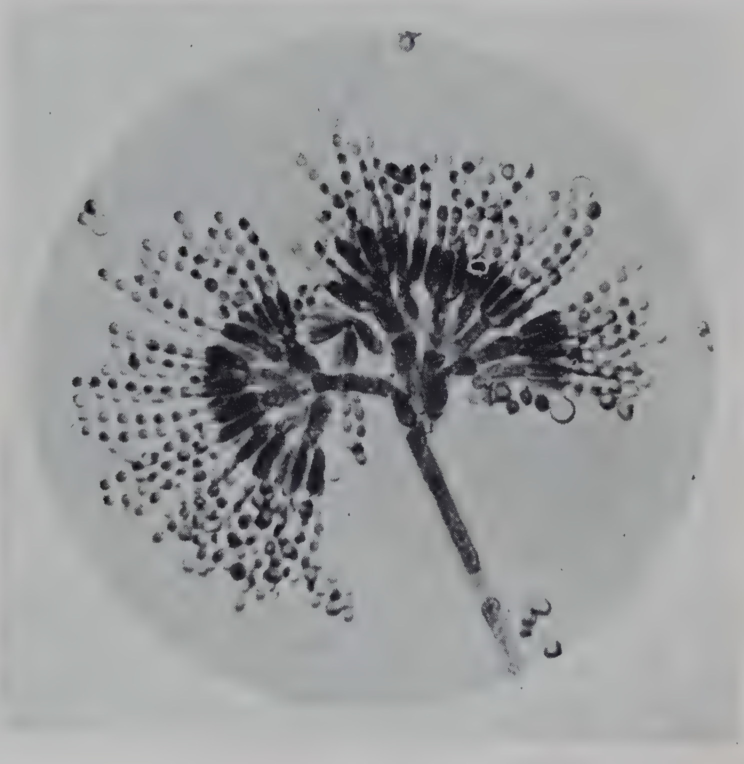


FIG. 128.—*P. brevi-compactum*—very large penicillus. × 600. Such are not uncommon, particularly in freshly isolated cultures.

a prostrate, matted layer of surface hyphæ. Colony colour is bluish green or somewhat dirty green, turning to greyish brown in age, with reverse more or less yellow to yellowish brown. All strains liquefy gelatine very rapidly. The type species is *P. brevi-compactum* Dierckx, synonym *P. stoloniferum* Thom. Some five or six other species have been described, but it is questionable whether most of the names are not synonyms of *P. brevi-compactum*. There is often a certain amount of variation between successive cultures of any one strain, the differences frequently being as great as between different so-called species. Occasionally freshly isolated cultures have an *Aspergillus*-like appearance, the conidiophores being long and stiff with large, complex penicilli and great masses of spores, but these characteristics are seldom retained in successive subcultures. Normally the spore chains are twisted and tangled, but examination of very young cultures will often show penicilli which are more or less divaricate, resembling those of *P. citrinum*. The conidiphores are smooth, all elements of the penicillus short and the spores are globose or almost so, 2·5–3µ in diameter. *P. brevi-compactum* is of common occurrence and world-wide distribution. It has been collected in many laboratories as an aerial infection; Thom's type strain was found on a rotting mushroom; it has been found on mildewed cotton goods and it is a common infection of stored maize, the consumption of infected grains being supposed at one time to be the cause of Pellagra.

When grown on liquid synthetic media containing glucose, particularly Raulin-Thom solution, a number of phenolic substances are produced (Clutterbuck *et al.*, 1932), detected by adding ferric chloride to the metabolism solution. In the early stages of growth the colour obtained is brown. Later it becomes redder and bluer and, after about three weeks, is an intense bluish crimson. Almost all species in the *P. brevi-compactum* series and, in the author's experience, *all* freshly isolated strains give the characteristic reaction, which is thus a useful confirmation of identification.

THE LANATA-TYPICA. The colony texture in this group is definitely floccose or woolly, at least in young cultures. Colonies usually spread at first as a white felted mass of hyphæ, which slowly becomes coloured as conidial heads ripen, the coloration starting at the centre and spreading outwards. Older colonies may be more or less velvety at the edges and in very old cultures the floccose central areas tend to die down to a compact, felted layer. Spore chains tend to form tangled masses rather than compact columns. The most important species in the group are the moulds used for ripening cheeses of the Camembert, Brie and similar types.

P. camemberti Thom, when grown on laboratory media, forms at first a white, densely floccose colony, slowly becoming pale greyish green. Typical penicilli are long, 70–90μ, and comparatively simple that is with few branches at each stage. The conidia are ellipsoid when young and gradually become globose, 4·5–5·5μ in diameter.

P. caseicolum Bainier, synonym *P. camemberti var. Rogeri* Thom, is a pure white form otherwise very similar to *P. camemberti*. It is used in the manufacture of cheeses which differ somewhat in flavour from those made with the true *P. camemberti*.

P. lanosum Westling may be regarded as the type of a series of forms which are fairly often encountered. Colonies are at first white, then grey-green and finally brownish, definitely woolly in texture. The conidia are smooth, or at most very slightly roughened, and not more than 3μ in diameter.

P. biforme Thom has been reported from a variety of sources and seems to be not uncommon. As a contaminant in cheese it is particularly objectionable because of its very strong, mouldy odour. Colonies are greenish grey, of varying degrees of floccosity on different media, often showing densely floccose patches mixed with almost velvety areas. Penicilli usually consist of two rami, with both metulæ and sterigmata few in the verticil and all parts smooth. Conidia are smooth, nearly globose, about 4μ by 3·5μ.

THE LANATA-DIVARICATA. As in the Velutina-Divaricata the penicilli appear as more or less compacted groups of monoverticillate heads, rather than as definite broom-like structures. The floccose character is not so well marked as in the last group, some species being, superficially, almost velvety. In addition, certain species tend to produce funiculose aerial hyphæ, so that there is no sharp line of division between this group and the next. The group includes three series of organisms which are common in soil and hence are found as infections of soil-contaminated materials.

P. janthinellum Biourge is the type of what Thom calls "the soil Penicillia." It is doubtful whether this and related species ought to be in this group at all, as they tend to produce ropes of hyphæ and thus seem to be allied to the *P. terrestre* series (see below). The name refers to the violet colour produced in reverse of colonies, most evident when these occur on plates of soil-extract agar. On Czapek agar the reverse colour is more usually yellow to orange, becoming reddish or faintly violet in age. Penicilli are typically divaricate, but

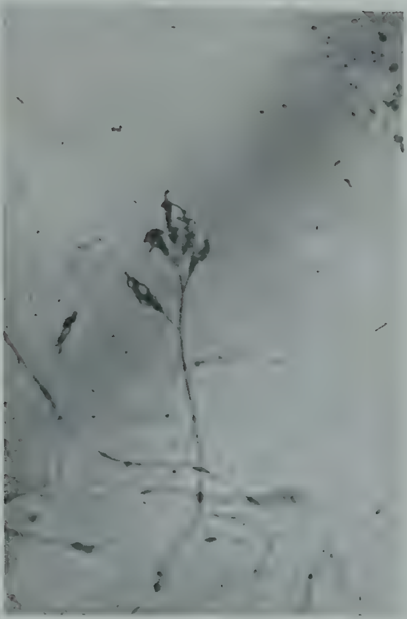


FIG. 129.—*P. janthinellum*—divaricate penicillus as seen in living culture. × 100.

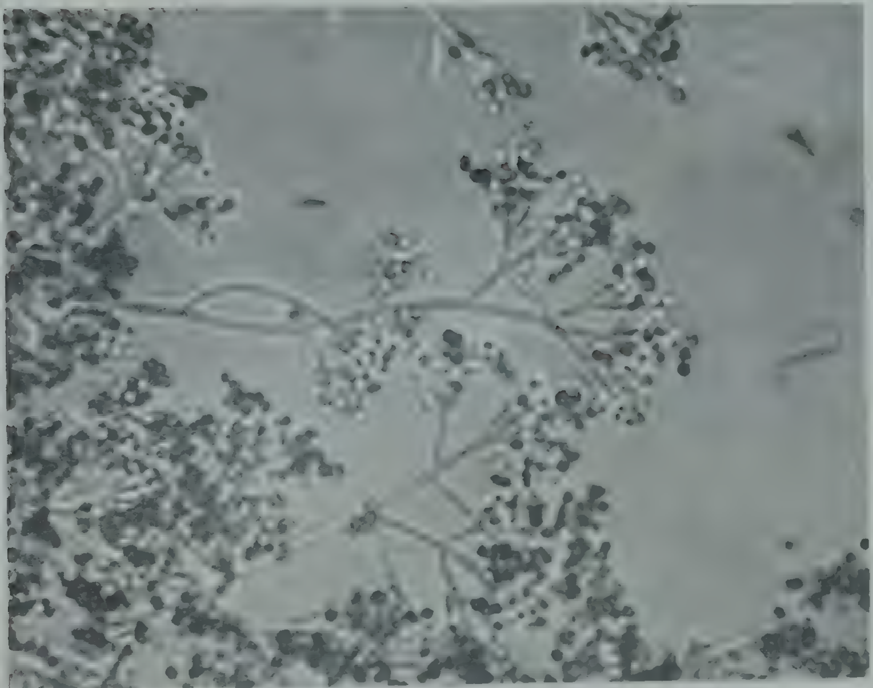


FIG. 130.—*P. nigricans*—penicillus. × 500.

the spore chains are divergent or tangled, not in columns (Fig. 129). Sterigmata have long, tapering points and the spores are globose or nearly so, about 2·5μ in diameter, smooth or slightly roughened.

P. canescens Sopp typifies a series of Penicillia which appear to be very common in English soils. Colonies form a thick woolly felt, bluish green or greyish green, with reverse at first yellow, passing to orange and orange-brown. The penicilli consist of a number of monoverticillate heads arising irregularly along the main conidiophores, bearing fairly compact columns of spore chains. Conidia are smooth, globose or nearly so, about 2·5μ in diameter. The odour is very strong and unpleasant.

P. Janczewskii Zaleski differs from *P. canescens* chiefly in less marked floccosity and in having rough conidia, 3–3·5μ in diameter.

P. nigricans Bainier is also commonly found in English soils. Colonies are definitely floccose and, in freshly isolated cultures, pure grey. After several transfers on laboratory media the colour tends to develop a slightly greenish tone, but is always more grey than green. Reverse deep orange to brownish orange; penicilli very irregular, consisting of short branches from long trailing hyphæ, or terminal on such hyphæ; sterigmata usually few in number on each branch, 7–8 by 2·5μ; conidia globose, spiny, about 3μ in diameter, in chains at first roughly parallel then tangled (Fig. 130).

THE ASYMMETRICA-FUNICULOSA. This group includes two series of species of quite different character.

Species in the series represented by *P. Daleæ* Zaleski are closely allied to the *P. Janczewskii* series in cultural and morphological characteristics and, like them, are soil organisms. The main difference is that the *P. Daleæ* series produce definite ropes of hyphæ, usually easily visible under a good hand lens and evident over the whole surface of the colony.

The second series of funiculose species, typified by *P. terrestre* Jensen, differs in that the penicilli are definitely broom-like and not divaricate. Strains allied to *P. terrestre* have been isolated from widely different sources, but there is good reason to believe that the real habitat is soil and that the name is, therefore, an apt one. Colonies are yellowish green to greyish green, floccose, with ropes of hyphæ evident, especially in fairly old cultures; reverse uncoloured or in pale greenish or tan shades. Odour is fairly strong and characteristic, in some strains like moist loam, in others somewhat sour. Penicilli are compact, with conidiophores definitely roughened and smooth globose spores, about 4μ in diameter.

THE FASCICULATA. This is a large group and includes a few species of considerable economic importance. The characteristic of the group is the aggregation of conidiophores into tufts, fascicles or definite coremia. Species which regularly produce coremia are easily recognized as belonging here, and the same may be said of certain strains whose colonies present a rough, granular appearance. There are, however, a fair number of species with a more or less velvety appearance when grown on ordinary laboratory media and which show obvious fasciculation only at the edges of colonies, or only when grown under special conditions or on special media. Most such species show a certain amount of mealiness on the surface of colonies, and examination of the edges of growing colonies will always reveal small tufts of conidiophores (Fig. 131). Another way of demonstrating fasciculation, in cases of doubt, is to pick off a small portion of a colony, from the edge or just inside it, immerse in a drop of alcohol on a slide, then mount in lactophenol without teasing out. Small bundles of conidiophores are thus readily observed.

P. gladioli Machacek is outstanding in this section because of its regular production of sclerotia. When grown at temperatures above 20°C. colonies consist almost entirely of whitish, buff or pinkish sclerotia, produced in concentric rings if the cultures are made in Petri dishes. At about 15°C. the growth is predominantly conidial, with sclerotia scarce. The colonies are then bluish green, with fasciculation evident. This species, as the name implies, is found as a cause of decay in Gladiolus corms.

The remaining species are divided into three groups according to their degree of fasciculation.

1. Fascicles and simple conidiophores mixed, but the latter predominating.
2. *Coremiella.* Fascicles and small coremia mixed with simple conidiophores, with fasciculation evident throughout the colony.
3. *Coremia.* Simple conidiophores absent or nearly so. Fructification mainly as definite coremia.

The first group is further divided into "Aeruginosa" with blue-green colonies, "Viridicata" with pure green or yellow-green colonies, and "Glauca" with dull or greyish green colonies.

In practice it will be found that this classification is not very satisfactory. Although the third group (Coremia) is fairly distinct, there is very little difference between some species of the first and second groups. Production of coremia depends largely on the substrate. *P. expansum*, for example, frequently produces large typical coremia in culture (Fig. 133) as well as on fruit, whilst some strains of *P. corymbiferum* (Group 2) are distinguishable from *P. cyclopium* (Group 1) only by colour of drops and of reverse of colonies. In addition, it is found by anyone examining numerous strains belonging to Group 1 that many of them do not fit well into any of the three sections. In particular, the blue-green section shades imperceptibly into the yellow-green, so that it is often impossible to decide definitely whether a strain should be called *P. cyclopium* or *P. viridicatum*. A further complication is introduced by the fact that some strains, which are definitely yellow-green when first isolated, tend to give colonies nearer to blue-green on continued cultivation, or, in some cases, are differently coloured on different media. It is manifestly impracticable to give specific names in sufficient number to cover all the variants, and it is probable that the best way of dealing with this group is to lump together all the blue-green and yellow-green strains with similar morphology as the *P. cyclopium-viridicatum* series.

Representatives of this latter group species are probably the commonest of all the *Penicillia*. They occur as saprophytes on every conceivable kind of organic material, they can be picked up almost anywhere by exposing Petri dishes to the air, and they are also found as parasites of the higher fungi and of various bulbous plants. Tulips in particular are liable to be attacked at the growing point, producing, as a result, distorted growth with blooms misshapen or lacking.

As representatives of the extremes of the series *P. cyclopium* Westling produces, on Czapek agar, colonies of a rich blue-green, tardily zonate, with reverse yellowish or pale pinkish buff, whilst *P. viridicatum* Westling is bright green, turning brown in age, with reverse yellow, orange or even reddish. In both species penicilli are fairly compact, complex with three or even four stages of branching, with rough conidiophores (the roughness slight in some strains and tending to be most marked in the yellow-green strains) and conidia smooth, globose or nearly so, mostly 3–3·5μ, but often up to 4μ in diameter (Fig. 132).

P. expansum (Link) Thom, the type species of the grey-green section, is best known as the cause of soft rot of apples and pears in storage, but it is not uncommon on many other substrata. The name covers a large number of strains which vary considerably in the freedom with which they produce coremia and in the degree of

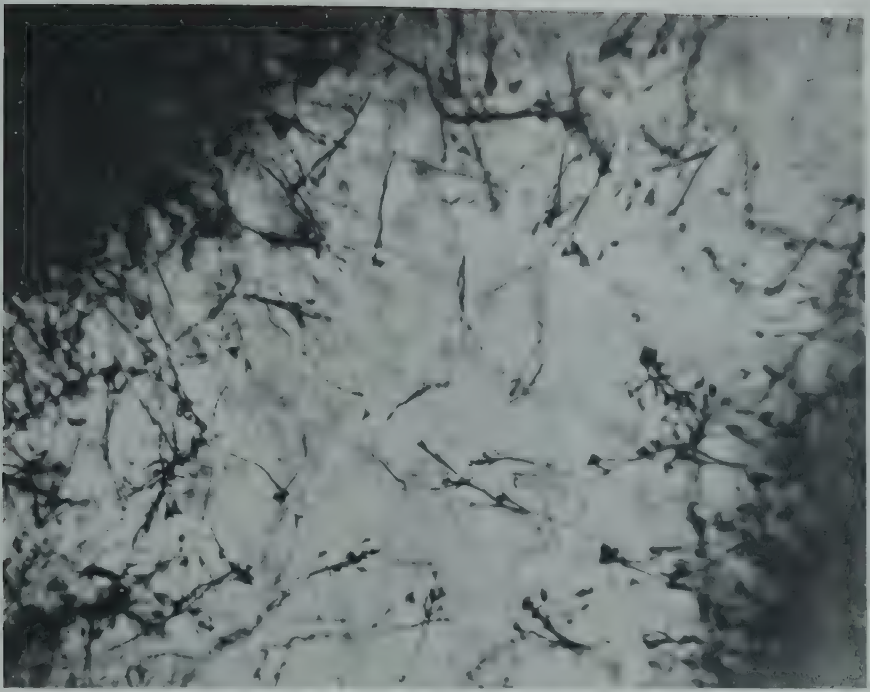


FIG. 131.—*P. brunneo-violaceum*—edge of colony in Petri dish, showing fasciculation. × 25.

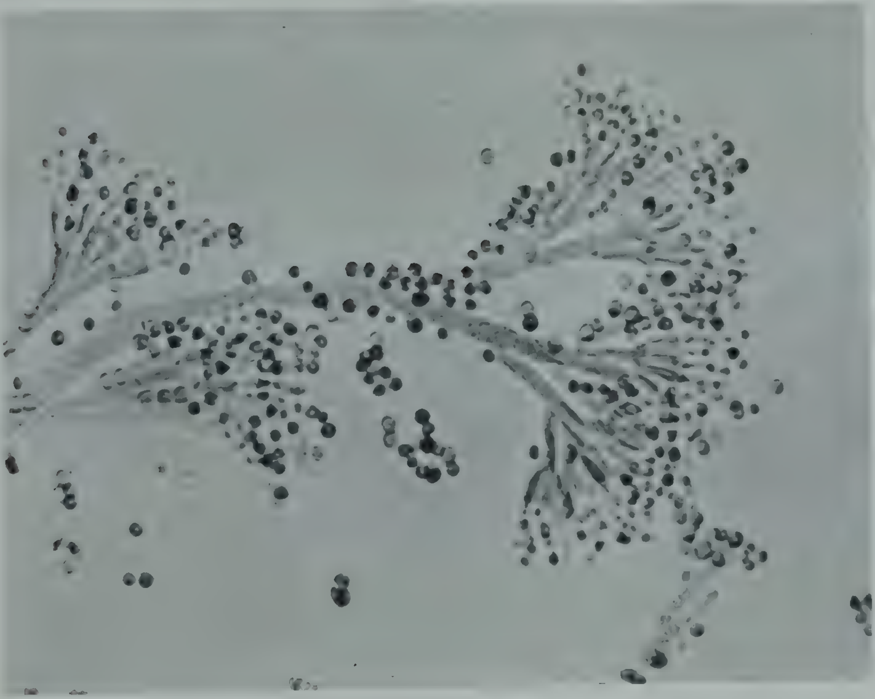


FIG. 132.—*P. viridicatum*—penicillus. × 500. *P. cyclopium* is very similar in appearance.

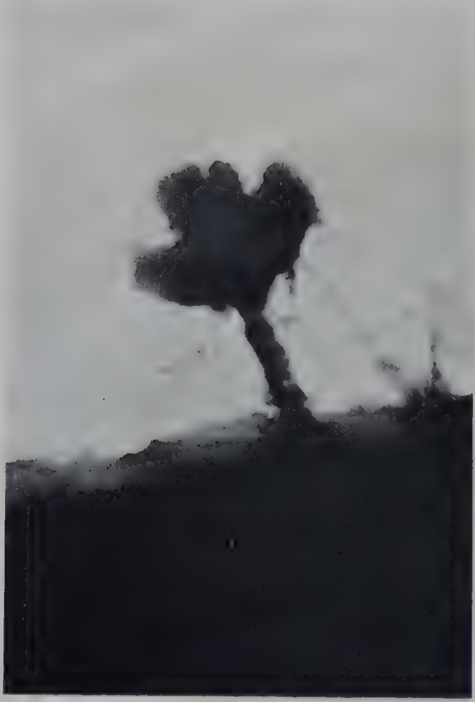


FIG. 133.—*P. expansum*—single large coremium on agar slope. × 25.

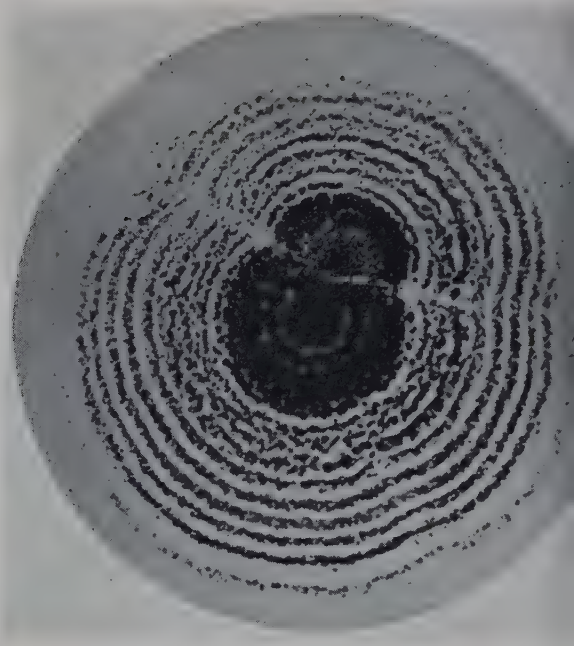


FIG. 134.—*P. expansum*—colony in Petri d showing well-marked zonation with rings small coremia. × 0·75.

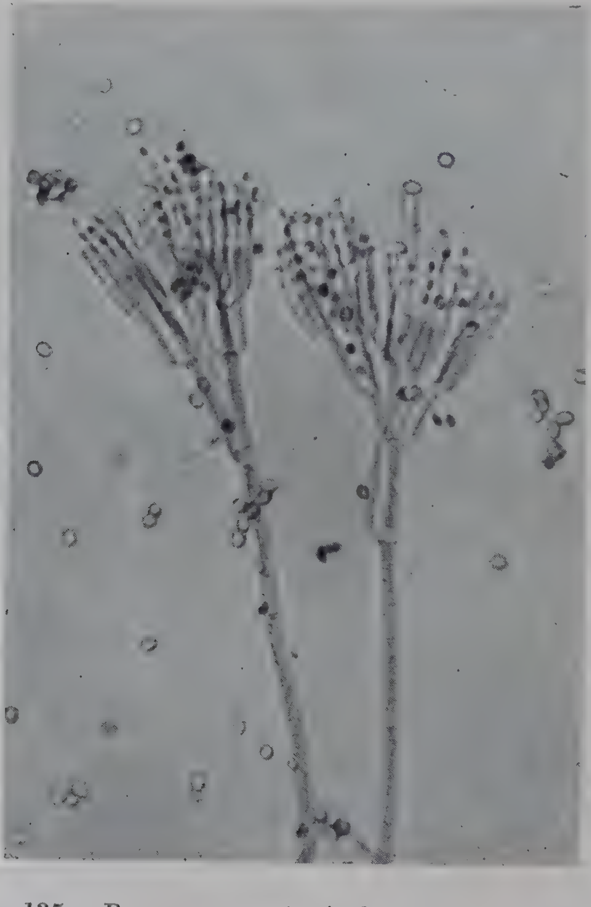


FIG. 135.—*P. expansum*—typical penicilli. × 500.

zonation of colonies when grown in Petri dishes. Colony colour is green or somewhat bluish green at first, rapidly becoming greyish green and then brown, with a broad white margin during the growing period. Colonies may be granular from the beginning or almost velvety, and showing fasciculation only in age. Fig. 133 shows a large coremium produced in an old culture, on an agar slope, which for the most part was almost truly velvety. The same strain, grown on a thin layer of agar in a Petri dish, is shown in Fig. 134. The concentric rings of coremia, with alternate sterile zones, are readily produced under similar conditions by many strains of this great group-series. The conidiophores, in fluid mounts, have a very characteristic appearance, the metulæ and sterigmata being comparatively few in number but well packed together (Fig. 135). Penicilli are usually 40–45μ in total length. The conidial chains are more or less in columns, the conidia being elliptical when young, 3·3μ by 2μ, becoming almost globose and 3 to 3·4μ in diameter, persisting in chains in fluid mounts.

P. urticæ Bainier (= *P. flexuosum* Dale) is of fairly common occurrence and is readily distinguished from *P. expansum*. Colonies are very obviously fasciculate, often forming definite coremia, of a colour which is more grey than green. The penicilli have three or four stages of branching, with all the elements very short and conidia about 2·8μ in diameter.

In this group Coremiella two species are of importance.

P. corymbiferum Westling is closely related to *P. cyclopium* and shows a similar tendency to parasitize bulbs. Colonies are blue-green to dark olive green, tardily zonate, with broad white margin and reverse deep orange, red or reddish brown, in some cultures fasciculate all over, with small feathery coremia (as described by Westling), in others merely granular, with no definite coremia. A very characteristic feature is the production of respiration drops of a deep orange or ruby red colour. The penicilli are compact, with rough conidiophores and conidia about 3μ in diameter.

P. italicum Wehmer is the cause of serious damage to citrus fruits. It is readily distinguished at sight from *P. digitatum* by the colour of colonies on the fruits, *P. digitatum* being dull yellowish green and *P. italicum* definitely blue-green. The two species are also distinguished by the type of rot produced. Fruits attacked by *P. digitatum* shrivel and dry up, whilst *P. italicum* produces a soft rot which rapidly reduces the fruit to a slimy pulp. *P. italicum* is also encountered fairly frequently in miscellaneous culture work. It is readily recognized by its production of curious prostrate coremi at the edges of colonies (Fig. 136) and by its characteristic conidiophores, with chains of *Oidium*-like young conidia (Fig. 137). Mos strains, when kept in cultivation for long periods, gradually los the typical colony habit, becoming floccose, with abundant aeria mycelium, and producing coremia only in age if at all.

The Biverticillata-Symmetrica

The name of the division refers to the type of penicillus commo to species placed here. In normal cases the conidiophore bears single whorl of metulæ, each bearing a whorl of sterigmata a approximately the same level, with the whole fructification closel packed (see illustrations). The sterigmata consist of narrow tube tapering to long points (usually described as acuminate), from whic the conidia are cut off as comparatively long, cylindrical segments becoming and remaining, when ripe, ovate, elliptical or pyriform. Th sterigmata and mode of formation of the spores are so distinctive tha they serve to place a species in this division even when, as occurs i some strains which fruit very sparingly, the majority of the penicilli d not attain full development and truly biverticillate heads are rare.

There are four sections of the Biverticillata: Ascogena, Coremigena, Luteo-virida and Miscellanea.

The Ascogena are not of common occurrence and the few species which have been described are of more theoretical than practical interest. The ascocarps are thin-walled, readily broken to liberate the asci, or with scarcely any wall. *P. avellaneum* Thom and Turesson lacks green colour entirely, colonies being of a pale sandy brown colour, with perithecia 300–600μ in diameter and ascospores ellipsoid, with walls pitted, 6·5–8·5μ by 4–5μ. *P. luteum* Zukal and *P. Wortmanni* Klöcker produce yellow ascigeral masses, with ascospores about 4·5μ by 3μ, with green or greyish green conidial areas and yellow colour in the mycelium. *P. spiculisporum* Lehman has definite perithecia, cream to pinkish, 0·4 to 2 mm. in diameter, and ascospores covered with minute spines.

Most ascosporic species tend, on continued cultivation, to lose their power of producing the perfect stage. Derx (1926) stated that *P. luteum* is heterothallic and that the loss of ascospore production is due to the segregation of the haplont forms when sub-culturing. Emmons (1935), however, has shown that none of the ascosporic *Penicillia* is heterothallic, but that a number of species show a gradual loss of fertility in artificial culture.

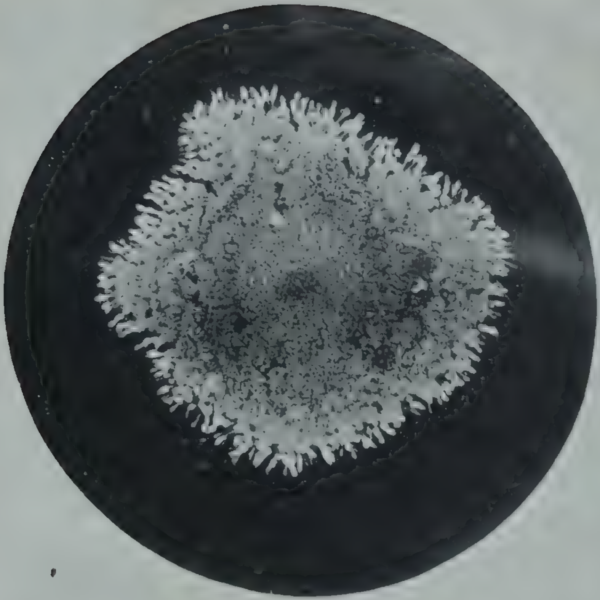


Fig. 136.—*P. italicum*—colony in Petri dish, showing prostrate coremia around the edge. Natural size.

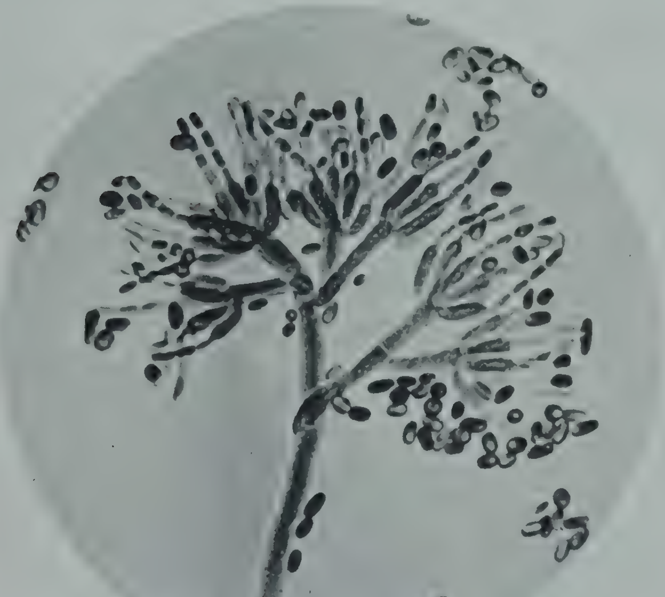


Fig. 137.—*P. italicum*—characteristic penicilli. × 500. Note the *Oidium*—like young conidia.

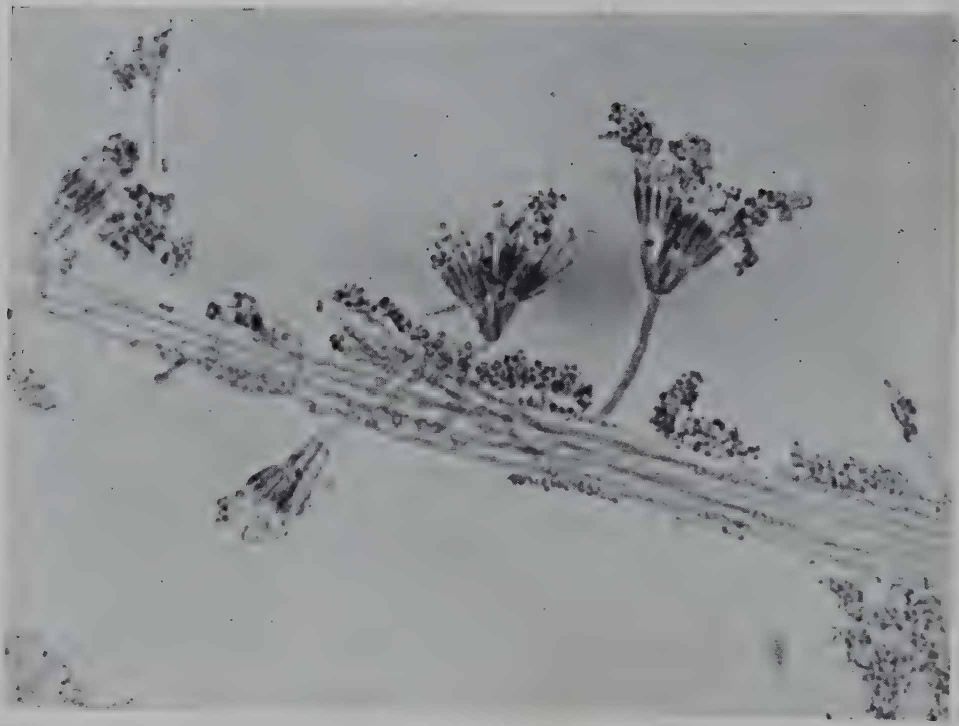


FIG. 138.—*P. funiculosum*—conidiophores arising from hyphal rope. × 500.

Of the Coremigena only one species, *P. Duclauxi* Delacroix, appears to be at all of widespread distribution. Cultures on media containing cane sugar, such as the ordinary Czapek agar, have a very striking and distinctive appearance. Masses of spiky coremia, several millimetres high, develop early, white at first, then greyish green. The mycelium gradually becomes yellowish whilst the medium slowly passes through yellow to an intense purplish red.

The Luteo-virida section includes a number of species of very common occurrence on all kinds of mouldy material.

P. funiculosum Thom is the type of a series of forms which produce ropes of fertile hyphæ, bearing, at right angles, short conidiophores with typically biverticillate heads (Fig. 138). The medium is usually slowly coloured an intense red, but strains are sometimes encountered which reproduce exactly the morphology of Thom's species but lack entirely the red pigment. Conidial colour varies in different strains from pale grey-green to deep green. Conidiophores are 20–80μ long with sterigmata 10–14μ, and the conidia are at first cylindrical, then elliptical, 3–4μ by 2–3μ.

P. luteo-viride Biourge has colonies of a rich green colour with margin yellow and reverse pink, orange and purplish red in indistinct zones and patches. Conidiophores are short, about 3μ in diameter, and the conidia ovate, 3–3·5μ by 2·3–2·5μ, usually slightly roughened.

P. rugulosum Thom is one of the commonest of all *Penicillia*. Colonies are a deep, rich green, in some strains almost velvety, in others definitely floccose, with reverse and edges of colony, but not the medium, deep yellow to bright orange in spots and patches. The conidia are ovate, about 3·5μ long, and are definitely roughened. An interesting occurrence of this species, and of a number of related strains, some of them approaching closely to *P. minio-luteum* Dierckx, is as parasites of the black *Aspergilli*. Hyphæ of the *Penicillium* twine up the stalks of the *Aspergillus* and smother the black heads with fruiting masses of a dark olive-green colour (Fig. 141, p. 206). Such parasitic forms cause trouble from time to time in factories where citric acid is manufactured by the mould fermentation process, attacking the mycelial mats of *A. niger* and causing portions to sink in the solution.

P. sanguineum Sopp occurs chiefly as a soil organism. Colonies are of a somewhat yellowish green colour, turning darker and with an overgrowth of yellow to orange hyphæ in age, markedly zonate, with rings of green separated by broad sterile interzones, and with reverse and medium deep orange to blood red. Conidial heads are more or less columnar and dense layers of conidia are forme breaking off in masses when cultures are disturbed.

P. luteum series. The name covers a series of non-ascospo forms, of fairly widespread occurrence, in which there is abunda production of floccose, not funiculose, aerial mycelium, more or le yellow in colour, with tardy development of greyish green conid areas and reverse yellowish to pale rose. The various strains sh considerable differences in intensity of colour and freedom of sporin but they are all supposed to be closely related to the ascospo species, *P. luteum* (Fig. 116).

P. purpurogenum Stoll shows less luxuriant aerial growth a greater freedom of spore production than the *P. luteum* seri The aerial hyphæ usually pass from yellow to red or purplish re whilst reverse and medium quickly turn a deep purplish red.

In the section Miscellanea Thom groups a number of species wi biverticillate penicilli which do not fit into any of the other sectio None of them is of particular importance and some are of ve doubtful relationship to the rest of the group.

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CHAPTER X

LABORATORY EQUIPMENT AND TECHNIQUE

The first essential in any laboratory experimental work involving microfungi is to obtain pure cultures; that is, to grow each species on a sterile substratum, known as the culture medium, free from admixture with any other organism and with suitable precautions to prevent subsequent contamination. Many fungi fail to grow characteristically in presence of other organisms and identifications are therefore rendered difficult. It is obvious also that studies of nutrition, of metabolic activity, or of the controlling effects of environmental conditions or of antiseptic substances, are without much value unless they are carried out with individual moulds and not with mixtures of species.

Methods of isolation, of culture and of study are, in general, similar to those used by bacteriologists but differ from them in detail. The methods described here are only a selection of those available but will probably be sufficient for preliminary essays in mycological studies, and the advanced worker and specialist can add to them as experience directs.

General Equipment

Tubes: Cultures for morphological study and for many other purposes are grown either in tubes or in Petri dishes. The tubes may, for most purposes, be ordinary test-tubes, but the special bacteriological test-tubes are much to be preferred. They are made of glass with a high resistance to chemical action and will withstand repeated sterilization. At the same time they are sufficiently strong mechanically to resist a certain amount of rough handling. The usual sizes are 6″ by $\frac{5}{8}$″ and 6″ by $\frac{3}{4}$″ or 6″ by $\frac{7}{8}$″. It is an advantage to have two sizes, the smaller without, and the larger with rims. The rimless tubes pack into baskets and storage boxes much better than the rimmed variety and are used for slopes (see below). The larger tubes with rims are used to hold medium for plating out, when the rim is a distinct help in pouring cleanly and the greater capacity makes thorough mixing of the contents easier.

Plugs: Tubes of culture medium, and tubes containing pure cultures, are always plugged with cotton-wool in order that any air which enters will be filtered from all contaminating organisms. The cotton-wool should be of the non-absorbent variety. It may be obtained in a number of different colours and these are useful for distinguishing the various culture media. A plug should project into the tube about an inch and should have a tuft outside the tube by which it can be extracted; it should fit accurately and tightly, but not so tightly that it cannot be extracted when gripped between any two fingers of one hand, and it should retain its shape, so that after withdrawal, it can readily be reinserted. There are various methods of making plugs by rolling and shaping before insertion, but an easy and quite satisfactory method is as follows: a strip about 2½″ wide is torn from the sheet of cotton-wool; from this a rectangular piece is torn, of a size that can be determined only by trial, and the edges are folded in to give a piece 2½″ long and of a width twice the diameter of the tube; this is laid across the mouth of the tube and its centre gently pushed in by means of a glass rod or the blunt end of an aluminium needle-holder. If the plug is definitely a tight fit when thus pushed in, it will be of the correct fit when the rod is withdrawn. The steaming which it receives during sterilization sets the plug more or less permanently to the shape of the tube.

Rectangular baskets of iron wire, galvanized or tinned, are used to hold tubes during sterilization and may be obtained of any convenient size.

Petri dishes are flat, circular, shallow glass dishes with perpendicular sides, provided with covers of the same shape, but of slightly larger diameter, so that they fit loosely over the dishes. The air which enters, and it must be remembered that fungi require a continual supply of air, is not filtered as in the case of plugged tubes. Owing, however, to the tortuous path it must take to enter the dish, suspended dust and spores are deposited outside and cultures are reasonably safe from contamination provided the dishes are handled with care and the air of the laboratory is not too heavily charged with infection. As an extra precaution, Petri dishes should, whenever possible, be stored and incubated upside down. Dishes may be obtained in various sizes but, for most purposes, the most convenient size is 10 cm. by 1·5 cm. They are sometimes made with plane, polished bottoms, but this is a refinement too costly for ordinary use.

General Apparatus: A certain amount of the necessary equipment is such as is to be found in any chemical laboratory. This include beakers and flasks of various sizes, measuring cylinders, pipettes funnels, Bunsen burners and tripods, balance and weights. It i assumed that the reader is sufficiently familiar with this type o apparatus for it to require no further mention.

A supply of needles and loops will be required for inoculation and the best are made from short lengths of stiff nichrome wire permanently fixed into long aluminium handles. Platinum wir cannot be set satisfactorily into aluminium as it becomes loose afte a short period, owing to the difference in coefficients of expansio of the two metals. Adjustable needle-holders are preferred by som workers, but they have too many hidden surfaces and require muc heating to ensure perfect sterilization, particularly when workin with liquid media. Occasionally a very stiff needle is required an a convenient form is triangular in section with fairly sharp edge that can be used for scraping. Fine pointed scissors and scalpel are useful for cutting up infected material preparatory to makin cultures and the ones made entirely of stainless steel are worth the extra cost, as they stand up to repeated sterilization without corroding. The list of tools should also include three pairs of forceps, one strong and blunt ended, one with fine points, and the third of the special type made for handling cover-glasses.

Some kind of support is necessary to hold a sterile needle whilst both hands are occupied in such tasks as manipulating plugs and labelling culture tubes. The porcelain, saw-tooth type of rack sold for the purpose is not really suitable, as it supports the needle-holder at the wrong end. The simplest support is a block of wood, 1– inches thick, or a large rubber bung, with a hole, just large enough to take the handle of the needle comfortably, bored through it. The needle stands upright in this, without serious danger of picking up infection. Such a support is invaluable when sowing large numbers of cultures. Other tools, which are used much less frequently, may be supported, after sterilization, in any way which ensures that the tips of forceps or the cutting edges of scissors and scalpels are not touching anything, e.g. resting on the lid of a Petri dish or a tripod.

Sterilization. When handling pure cultures it is necessary to sterilize all tools, utensils and culture media. Metal tools are sterilized by heating in a Bunsen flame, needles until red hot, cutting tools at a somewhat lower temperature to avoid loss of temper. Dry glassware, such as tubes, flasks and Petri dishes, are sterilized by dry heat, and a capacious air-oven should be available capable of being maintained at a temperature of about 160° C. Three hours' heating at this temperature is sufficient to sterilize anything. Petri dishes are preferably packed in boxes before heating, and allowed to cool and remain therein until required for use. Special boxes of sheet iron or copper are made for the purpose, but they are expensive, and the rectangular tins in which biscuits are sold serve the purpose just as well. Culture media are sterilized by steam, either at atmospheric or higher pressure, so as to avoid change of concentration by evaporation. The Koch type of steamer, designed for sterilization at ordinary pressure, is a tall copper vessel, cylindrical in the usual style, the lower portion of which serves as a water-bath and is fitted with a gauge and sometimes with a constant level attachment. A perforated shelf, fixed a little distance above the water-level, serves to hold apparatus. The lid is provided with a tubulure to take a thermometer and to act as a steam outlet, and the whole outside of the steamer, except the bottom, is lagged with felt. It is normally supplied with a stand of sheet iron and a ring burner. The internal height of the Koch steamer should be sufficient to accommodate a litre flask and a funnel of 7″ diameter supported above it. Larger sizes of steamers to hold several baskets of tubes or a number of flasks, are usually rectangular in shape. Where much sterilization of media has to be done it is an advantage to have an autoclave in which steam is generated under pressure, usually 10 to 20 pounds to the square inch, and the time required for sterilization thereby much shortened. Full particulars of autoclaves, as well as of Koch steamers, may be obtained from the catalogues of laboratory furnishers. When heating culture media it is advisable to cover baskets of tubes and the mouths of flasks with grease-proof paper to prevent the plugs being wetted by drips from the lid of the sterilizer. The paper caps should be removed as soon as the vessels are taken out so that the plugs can become thoroughly dry.

Sterile graduated pipettes are frequently required for sowing liquid culture media and for making adjustments to sterile media. They are plugged at the mouthpiece ends, pushing the plugs well into the tubes, wrapped separately in brown papers so that they are completely enclosed, and sterilized by dry heat, at a temperature not exceeding 130° C. for five to six hours. At a higher temperature the paper is charred and becomes useless as a protection.

Tubes for use in aeration experiments are fitted with the necessary rubber bungs, ready for insertion into flasks or tubes, every open end is plugged with cotton-wool and the whole is wrapped in grease-proof paper and sterilized in the autoclave.

Another matter, which is not strictly concerned with sterilization in the usual sense of the word, may be mentioned here. It is sometimes necessary to sterilize the air of a laboratory when it becomes so heavily charged with infection that cleanly work is difficult. A quick way of partial cleansing of the air is to spray thoroughly with a 2 per cent solution of thymol in spirit, using an instrument which gives a fine, mist-like spray. Thymol is a very efficient fungicide and the alcohol ensures that any spores with which it comes in contact are properly wetted. A thorough method of sterilization, to be used preferably during a week-end, is to charge the air of the room with formaldehyde. A heap of sodium permanganate (potassium permanganate serves as well but is more expensive) is placed on an iron tray on the floor and commercial formalin is poured on in sufficient quantity to wet the heap through. A rapid evolution of gaseous formaldehyde occurs and cleanses the air quickly and efficiently. It is necessary to see that all windows and ventilators are closed before commencing operations and also, when the formalin has been added, to remove oneself quickly, shut the door and see that no one enters the room for at least twenty-four hours. Another good method is to seal up all openings and blow live steam into the room but, obviously, this practice can be adopted only when the fittings of the laboratory are made to withstand it. In some institutions, where the special requirements of microbiological work have been considered at the time of building, the culture laboratory has walls of glazed brick, benches of stone or tiles, and metal doors and window-frames which can be made airtight, the air being steamed periodically as a matter of routine.

Incubators. In order to obtain rapid and characteristic growth of many species of fungi, it is essential to grow them at temperatures above that of the average laboratory, and an incubator, in which a steady, predetermined temperature is maintained automatically and indefinitely, is absolutely necessary. In physiological work it is often desirable to study the effect of temperature on growth and this, of course, unless the work is to be prolonged unduly, involves the use of a series of incubators set for different temperatures. The optimum temperatures for different fungi cover a wide range, but the great majority of common moulds grow best at about 25°C. In purchasing an incubator for general use it must therefore be specified that the regulating capsule supplied is the correct one for this temperature, since the usual bacteriological incubator is sent out supplied with automatic control for 37°C. It is often better to have two incubators of moderate size rather than one large one so that, if occasion demands, a certain amount of study of temperature relations can be carried out without disturbing cultures which are best kept at 25°.

Culture Media

A great many different culture media are used for the propagation and study of fungi, but the majority have been designed for some special purpose, such as the securing of optimum growth of a particular species or for determining the availability of specific substances for mould growth. The only ones which will be noted here are such as are in general use for isolation, propagation and morphological studies. They can be added to or altered as experience suggests.

Mycological media differ in several respects from the media used by the bacteriologist. The latter are usually slightly alkaline in reaction, whereas most fungi prefer a slightly acid medium and many species can tolerate a fairly high acidity. Bacteriological media commonly contain protein as a source of carbon and nitrogen, whilst the majority of the substrates used by the mycologist have carbohydrates as a source of carbon, and nitrogen is supplied in an inorganic form, as nitrates or ammonium salts. The chemical elements which are known to be essential for growth of fungi are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, magnesium and iron. It is probable that, in certain cases, traces of other elements are necessary, but it is seldom that such need to be provided specifically. Many vegetables and vegetable extracts are eminently suitable as culture media without any addition, or by addition of sugar only, and a few such are very useful for isolation of species and maintenance of stock cultures. Some workers prefer to use purely synthetic media exclusively, as these are of constant composition and therefore strictly comparable when made in different laboratories. There is much to be said for this practice, particularly in taxonomic work. The type of growth shown by any particular species may vary considerably according to the medium used and, in consequence, it is of great importance, when describing new species, to record observations of cultures made on media which can be prepared in identical form by any other worker. In biochemical studies of fungi such synthetic media are used almost exclusively.

Culture media may be either solid or liquid. The term solid media is used in two different senses, to mean either actual solid substances, usually portions of roots, stems or seeds of plants, or aqueous solutions made into jellies by the addition of gelatine or agar. Agar may be considered to be a solidifying agent pure and simple, since it is utilized as a source of carbon by very few species. Its most useful property is the great difference in temperature between its melting-point and solidifying-point. At the concentrations commonly used agar media do not melt till the temperature exceeds 95° C. and can therefore be used for incubating cultures at high temperatures. The molten medium does not resolidify until the temperature falls below 40°, so that infected material or portions of mould growth may be mixed with the medium, before pouring into Petri dishes, without any danger of killing the spores. Gelatine, on the other hand, serves as an excellent culture medium for many moulds without the addition of any other nutritive substance and cannot, therefore, be considered as an inert support when used to solidify any other medium. Gelatine media are readily liquefied on warming and cannot be used at temperatures above 30° at the outside. They are also liquefied by the enzyme action of many fungi, and the chief use of such media is for diagnostic purposes based on the presence or absence of this liquefying property.

Solid media are easier to handle than liquid media and are to be preferred whenever requirements permit. Liquids are used chiefly in biochemical work, when it is often necessary to determine the course of metabolism by analysis of the medium or to isolate some specific product of fungal activity.

Preparation of Agar Media. Agar-agar is a carbohydrate obtained from certain species of seaweeds. It may be purchased as strips, of a pale brownish colour, or as a fine greyish powder. The strip form is preferred by some workers, as it is said to give a more transparent medium, but it requires prolonged soaking before it can be dissolved whereas the powder dissolves very readily and is more convenient in use. The amount of agar to use varies with the type of culture fluid. Media which are not strongly acid seldom require more than 1·5 per cent, as this amount gives a jelly which is firm without being too solid and having little tendency to crack. With media which have a distinctly acid reaction it is necessary to use 2 per cent or even more, since agar is readily hydrolysed by heating with acid and thereby rendered incapable of setting.

The method of preparation is very simple if powdered agar is used. The required amount of powder is added to the culture fluid, well distributed by shaking, and the mixture heated to boiling-point. Heating may be carried out in the steamer but is quite safely performed over a Bunsen burner if the liquid is constantly stirred to prevent sticking on the bottom of the vessel. The agar is completely dissolved by the time the temperature reaches boiling-point. For most purposes it is unnecessary to filter the agar medium, the slight cloudiness, which is nearly always present, being no disadvantage, but for some purpose, such as making single spore cultures, involving microscopical examination of agar plates, it is undoubtedly preferable to have a perfectly clean medium. Filtration through ordinary filter paper is impracticably slow, even with the aid of a hot-water funnel or by carrying out the filtration in the Koch sterilizer. The special "Chardin" type of paper, a very thick soft variety, permits of more rapid filtration but does not give a perfectly clear medium. The best method is to tear up sheets of "Chardin" paper into small pieces, place these in very hot water and shake vigorously until a pulp is obtained, pour this on to a Buchner funnel and suck dry to form a fairly thick pad, wash with hot water until the funnel and flask are heated through, and immediately filter the hot medium with suction. Filtration is very rapid and a beautifully clean product results. When the agar is dissolved, and after filtration if this is carried out, the medium is filled into tubes, which are then plugged and sterilized, either by autoclaving for twenty to thirty minutes at 15 pounds' pressure, or by steaming for an hour on two or three consecutive days.

Preparation of Gelatine Media. A good-quality sheet gelatine—Coignet's "Gold Label" is the best—is cut into small pieces, covered with the required amount of water or liquid medium and allowed to soak for several hours or overnight. The vessel is then heated until solution is complete and the medium is tubed and sterilized by steaming for thirty minutes on three successive days. Prolonged heating destroys the power of solidification. This process of intermittent sterilization is always used for media which are unable to withstand continuous heating. The theory of the process depends on the fact that vegetative structures are more readily killed by heat than are the spores. The first short cooking destroys most of the vegetative growth but may not kill all the spores. These, however, being in favourable situation for growth, and being already swollen by the moist heat, germinate rapidly. The second heating destroys the new growth and the third day accounts for any spores whose germination has been delayed. The strength of gelatine media needs to be varied somewhat according to the season. A 10 or 12 per cent solution will give a firm jelly in all but very hot weather, and 15 per cent is sufficient for such occasions.

VEGETABLE MEDIA

1. Potato Plugs. Large, healthy potatoes are scrubbed and washed in water. In some laboratories they are next sterilized by soaking for about twelve hours in a 0·1 per cent solution of mercuric chloride or in a dilute permanganate solution, but if the potatoes are sound, this is unnecessary. The tubers are peeled and again washed, and then cylinders, 2½ to 3 inches long, are cut out with a cork borer. The diameter of the plugs should be only very slightly less than the internal diameter of the culture tubes. The cylinders are cut in two, lengthwise and diagonally, and the halves are put into the tubes, with the thick ends downwards and resting on pieces of wet cotton-wool. The latter is to prevent the surface of the potato from drying out too rapidly. The tubes are plugged and sterilized by autoclaving at 20 pounds' pressure for twenty minutes on two successive days.

Potato plugs are sometimes useful for bringing about sporulation in obstinate cultures, but like other similar media, they have the disadvantage that only the surface of the colony is seen and characteristic features, which are noted when the reverse side of a Petri dish is examined, are missed.

2. Other Solid Vegetables. Carrots may be used as plugs, made in the same way as potato plugs, or may be cut into slices. Beans are used whole, whilst stems of various plants are cut into short lengths. Otherwise, the details of preparation are the same as for the potato medium.

3. Vegetable Decoctions. Extracts of potatoes, beans, carrots and prunes are the decoctions most commonly used. Fifty grams of prunes or dried beans, 100 g. of carrots, or 200 g. of peeled potatoes cut into small pieces are boiled for an hour in 1 litre of water. The liquid is strained through fine muslin, or filtered, and solidified, if required, with 1·5 to 2 per cent of agar. The medium is tubed and autoclaved for twenty to thirty minutes at 15 pounds. The amounts given are average figures and may be varied according to requirements.

The prune medium, without additions, gives good growth of most moulds, but the bean, carrot and potato extracts lack soluble carbohydrate and are improved by the addition of 1·5 to 3 per cent of glucose or cane sugar. Potato-dextrose agar, as used in many mycological laboratories, contains 2 per cent of glucose. The carrot medium, with addition of 2 per cent cane sugar, is an excellent medium for the majority of the Dematiaceæ and for species of *Fusarium*, and has been found to induce sporulation of certain Ascomycetes which grow scarcely at all on other media in common use.

4. Wort. Unfermented sweet wort from a brewery is diluted to a specific gravity of 1·05, heated in the autoclave for half an hour at 10 pounds' pressure and filtered hot. If no autoclave is available the wort may be heated for an hour in the steamer, but in this case it will be found that subsequent sterilization causes a further precipitate to be thrown down, whereas, if the wort is first heated to a temperature above 100° C. and sterilization is carried out in the steamer, this does not occur. Wort-agar is made by the addition of 2 per cent of agar and the medium, being distinctly acid, should not be over-sterilized.

In some districts wort is difficult to obtain and recourse must be had to commercial malt extract. This is not a complete substitute, since it is not practicable to make a solution which is as rich in nutrients as natural wort. A medium which has been found to be completely satisfactory is made by adding 5 per cent malt extract, 3 per cent cane sugar and 2 per cent agar to Czapek's solution (see below).

Wort media give very vigorous and characteristic growth of the great majority of species and are very useful for isolation of species and for stock cultures of some of the more delicate and slow-growing fungi. Some species, however, tend to produce an undue amount of mycelium at the expense of spore-bearing structures, and these are best kept on a less rich medium. A still richer medium, much favoured by some mycologists, is made with 10 per cent of gelatine instead of agar. One interesting feature of wort is that it is very heavily buffered and has no tendency to become alkaline, as many synthetic media do, when the food material is becoming exhausted. Many of the green moulds, *Aspergilli* and *Penicillia*, turn to a dirty grey or brown shade on such media as Czapek-Dox but retain their green colour for months on wort.

5. Yeast Extract. This is not used alone but can sometimes be added to other media with advantage. With some species there is little effect, with others stimulation of vegetative growth, whilst the effect on a few species is to induce more abundant sporulation. Pressed yeast, 200 g., is autoclaved with a litre of water. The suspension is filtered hot, and again after cooling, and made up to the original volume. The amount of extract to use in any particular case, and the best medium in which to incorporate it, must be found by experiment.

6. Corn-meal Agar. Consists of corn meal (ground maize), 60 g.; agar, 15 g.; water, to 1 litre. Mix the corn meal to a smooth cream with the water, simmer for 1 hour, filter through cloth, add the agar and heat till dissolved, make up to original volume and sterilize, preferably in the autoclave for thirty minutes at 10 pounds pressure.

This medium is widely used and is especially valuable for stock cultures of very many fungi, particularly of most of the dark coloured moulds.

SYNTHETIC MEDIA

7. Plain Gelatine. Simply a 10–15 per cent solution of gelatine in water, prepared as described above.

8. Sugared Gelatine. The addition of 1 to 3 per cent of cane sugar, or glucose, to plain gelatine gives a medium which usually induces more vigorous growth than the unsweetened medium. It serves for determination of liquefying power in many species which grow very poorly in the absence of sugar. The method of preparation is obvious.

9. Raulin's Medium. The first attempt to compound a rational, synthetic medium was by Raulin (1869), who analysed the ash of *Aspergillus niger* and, on the basis of his analysis, made a medium which he used for biochemical studies of this species.

Sugar candy	70 gr.
Tartaric acid	4 g.
Ammonium nitrate	4 g.
Potassium carbonate	0·6 g.
Ammonium phosphate	0·6 g.
Magnesium carbonate	0·4 g.
Ammonium sulphate	0·25 g.
Zinc sulphate (crystals)	0·07 g.
Ferrous sulphate (crystals)	0·07 g.
Potassium silicate	0·07 g.
Distilled water	to 1500 c.c.

The only difficulty in making up this solution is to dissolve the silicate, but it is hard to see what useful purpose it serves and it can certainly be omitted without detriment. The reaction is strongly acid, *p*H 2·9, and it is almost impossible to make an agar medium which will set. Growth of *Aspergillus niger*, for which the medium was designed, and of a few other species is good, but a great many moulds fail to grow characteristically. Raulin's solution is of interest chiefly because it forms the basis of many other media, some of them of great value.

10. Raulin-Thom Solution. Thom and Church, in The Aspergilli (1926, p. 40), make a curious mistake in quoting Raulin's medium and the mistake is repeated in Thom's Penicillia (1930, p. 36). Although it is mentioned in the text that the solution includes ammonium nitrate, the formula given omits this salt and substitutes ammonium tartrate. The result is an extraordinarily interesting medium for the purpose of biochemical work and one which gives good growth of most common moulds. It is less acid than the original Raulin's medium the *p*H being about 3·9.

The medium is not easy to make up, as magnesium tartrate tends to separate and does not readily re-dissolve. The following is a satisfactory method. The tartaric acid, finely powdered, is dissolved in about 500 c.c. of warm water and the $MgCO_3$ is dissolved in this solution. The ammonium tartrate is added and the liquid stirred till it is all in solution. Next the sugar is dissolved in the mixture. The potassium carbonate is dissolved separately in 100 c.c. of water and added to the rest. The ammonium, zinc and ferrous sulphates are also separately dissolved and added. Finally, the phosphate is dissolved in 500 c.c. of water and added to the main solution with constant stirring, and the medium is made up to correct volume. If properly compounded, the medium should be perfectly clear and of a faint yellow colour which becomes somewhat deeper on sterilization.

11. Neutral Raulin-Dierckx Medium. This is another interesting modification of Raulin's medium, designed by Dierckx for use with Penicillia and strongly recommended by Biourge (1923, p. 43). It certainly induces an extraordinary range of colour production in species of Penicillium and, in addition, often gives typical growth of coremiform species, in this and other genera, when other media fail. The method of making up the solution is given by Biourge as follows:

1. Dissolve 0·40 g. of $MgCO_3$ with 0·71 g. of tartaric acid in 100 c.c. of water.

2. In 800–900 c.c. of distilled water dissolve saccharose 46·6 g.; NH_4NO_3 2·66 g.; ammonium phosphate 0·40 g., K_2CO_3, 0·40 g.; $(NH_4)_2 SO_4$ 0·16 g.; $ZnSO_4,7H_2O$ 0·04 g.; $FeSO_4 \cdot 7H_2O$ 0·04 g.
3. Add 66·7 c.c. of solution (1) and make up to 1000 c.c.

In a note on another page (p. 37) Biourge says, "Take 0·27 g. $MgCO_3$ and 0·40 g. tartaric acid. Bring together in a small mortar until clear, then dilute at once considerably to stop crystallization"; the rest of the formula being as above. He adds, "The small amount of precipitate can be ignored if distributed equally in containers." For a solid medium Biourge recommends the addition of 100 g. of gelatine.

12. Czapek's Solution. Raulin's solution and its direct modifications are unnecessarily complicated. Czapek's medium is an attempt to supply all the elements necessary for mould growth without duplication. The basal salt solution is here given as modified by Dox (1910) and Thom and Church (1926), p. 39).

Sodium nitrate	2·0 g.
Potassium chloride	0·5 g.
Magnesium sulphate $MgSO_4.7H_2O$	0·5 g.
Ferrous sulphate, $FeSO_4.7H_2O$	0·01 g.
Potassium phosphate, K_2HPO_4	1·0 g.
Water	to 1000 c.c.

The basal solution may be used with various amounts of sugar, with different sugars, or with other sources of carbon. The most usual addition for general taxonomic work is 30 g. of cane sugar, for optimal growth of *Aspergillus glaucus* 200 g. cane sugar, and for biochemical work 50 g. of glucose. If glucose is used it is necessary to make up the medium without phosphate and add the latter in concentrated solution after sterilization; otherwise the medium becomes brown and turbid on heating. The reaction is neutral and quite an appreciable amount of magnesium phosphate is precipitated, indicating that the medium might with advantage be slightly modified. Many workers make up the solution with acid potassium phosphate, KH_2PO_4. The reaction is then definitely acid, the *p*H being about 4·2, but even so, there is still a small amount of precipitation on sterilization. With the acid phosphate there is no browning of glucose on heating. The acid medium gives rather better growth of most moulds than the neutral form.

In compounding the medium it is best to dissolve all the salts, except the phosphate, in about half of the water, add the sugar, dissolve the phosphate separately and add to the rest, finally making up to correct volume.

Czapek agar, perhaps the most generally useful of all solid media, is made by the addition of 1·5 per cent of agar. It is recommended by various authors for taxonomic studies, by Thom and Church for *Aspergilli* (1926, p. 39), by Thom for *Penicillia* (1930, p. 42) and by Waksman for Actinomycetes (1931, p. 279).

TYPES OF CULTURES

Slopes. Cultures which have to be stored are almost invariably made on agar slopes in tubes. For making slopes in tubes of $\frac{5}{8}''$ diameter the amount of agar medium should be 5–6 c.c. The medium is sterilized in the tubes in the ordinary way and, whilst the agar is still hot and molten, the tubes are inclined at such an angle that the medium forms a layer of decreasing thickness from the bottom of the tube to within about an inch of the plug. Laying the tubes on the bench with the plugged ends supported on a glass rod half an inch in diameter gives about the right amount of slope. The object of sloping the agar is to provide a relatively large surface, on which the progress of growth of a colony can be watched far better than on a level surface of agar in a comparatively narrow tube. Incidentally, the varying thickness of the layer of medium often reveals interesting cultural characteristics, the type of growth at the shallow end showing marked differences from that at the deep end.

The method of sowing slopes is to hold the tubes by their lower ends between the thumb and first finger of the left hand, with the plugs on the palm side of the hand and pointing slightly downwards. Three is the maximum number of tubes which can be conveniently held, allowing of two transfers being made in one operation from a tube culture, or three cultures being sown from infected material. A needle is sterilized by heating to redness in a Bunsen flame. Whilst it is cooling the plugs are removed, one at a time, by the right hand, using a twisting motion, and placed between the other fingers of the left hand so that they are held by their tops only. The Bunsen flame is played round and into the mouths of the tubes, an operation known as "flaming," until the glass is too hot to touch. The needle is then used to pick up a few spores or a fragment of mycelium from the parent culture and this is deposited, as rapidly as possible and without being allowed to come in contact with the hot glass, on to the fresh agar surface. The mouths of the tubes are again flame and the plugs inserted. Some workers flame the tubes again, afte insertion of the plugs, but this is not necessary unless the plugs hav been out of the tubes for an appreciable time, and it causes the top of the plugs to become charred and messy to handle.

When large numbers of cultures of a single species are require the best method, provided the parent culture is sporing freely, is t use a spore suspension. A small amount of spore material is transferred, with the usual precautions, to about 1 c.c. of sterile wate contained in a very small test-tube, the tube is shaken to distribut the spores and is then supported, as nearly horizontal as possible in such a way that the mouth can be flamed periodically during th sowing. The inoculations are made with a wire loop, a tiny drop o the suspension being transferred to each fresh tube of medium Loops of standard size, made of either platinum or nichrome wir and either unmounted or fixed in handles, can be purchased but ar readily made from the ordinary wire needles.

Cultures on Liquid Media. When sowing on to liquid media in tubes the method is the same except, of course, that the tubes cannot be held with mouths pointing downwards. They should be held as nearly horizontal as possible without getting the liquid on to the hot glass near the mouth, in order that spores of stray moulds cannot fall directly on to the surface of the medium but will fall on the hot glass and be killed.

Plates. For morphological studies microfungi are grown in Petri dishes (commonly known as "plates"), as well as on slopes. Dishes planted with single colonies are useful for determining rate of growth and colony characteristics such as zonation and sectoring, whilst plates containing several colonies are more suitable for microscopical examinations. Many species form dense, opaque felts of mycelium so that the only part of an isolated colony which can be examined by transmitted light is the extreme edge, and this usually shows no ripe fruiting structures. In a dish containing several colonies it is usually found that, along the edges where these approach each other, narrow sterile zones are left and spore-bearing organs can be clearly viewed as they hang over these gaps.

The usual 10-cm. Petri dish requires approximately 12 c.c. of medium to give a layer of adequate thickness, and, if comparative cultures of different species are required, the amount of medium per dish should be fairly accurately standardized. The medium is filled into tubes in correct amounts and sterilized therein. Before pouring into plates it should be allowed to cool down to about 45°C., best by leaving the tubes for a few minutes in a water-bath maintained at this temperature, as very hot medium gives off water vapour, which condenses on the cool lid of the dish and then drips back on to the medium. Each tube, as it is lifted from the water-bath, is held in a sloping position whilst the plug is removed and the mouth flamed, and then the medium is poured gently into the dish whilst one edge of the cover is raised as little as is necessary for the purpose. After replacing the lid the dish is gently tilted to spread the medium and then left to stand on a level surface until the agar has set. Wherever possible it is best to store Petri dishes in the inverted position, both before and after sowing, as this minimizes the chance of infection, and when sowing plates, or handling them for examination, every care must be taken to avoid exposure of the medium more than is absolutely necessary, since protection by flaming, as in the case of tubes, is impossible. With dishes in the normal position it is not easy to sow colonies in predetermined positions, the inoculating needle often leaving a trail of spores right across the surface of the medium. If the dish is inverted, then lifted out of its cover, and the needle approached and receded from directly below, it will be found that very few stray colonies will appear. Another method of sowing single colonies is to inoculate with a loopful of a spore suspension.

Slide Cultures. For the study of some species which produce very small and fragile sporophores the only satisfactory method of making preparations for microscopical examination is by means of slide cultures. There are two easy ways of making cultures on microscopic slides. If the fungus spreads close to the substratum it is grown on a very thin layer of agar spread on the slide. The agar medium must be clean and transparent. A piece of glass rod or, better, a strip of metal not more than 1 cm. in width is bent twice at right angles, in such a way that it will fit into a Petri dish and support a slide clear of the bottom. The dish, with the support and slide in position, is sterilized by dry heat. A drop of melted agar is poured on the centre of the slide whilst the latter is still warm and spread as evenly as possible by means of a bent glass rod. If it is desired to study the germination of spores, a little spore material is mixed with the agar before pouring on to the slide, but if spore production is to be observed, it is better to plant the medium at two or three points. About 10 c.c. of a sterile 20 per cent solution of glycerol is poured in the bottom of the dish. This keeps the thin layer of agar moist, but not wet, as it would become if pure wate were used. The slide is sufficiently near to the lid of the dish to allow observation of the progress of growth with a low-power objective; i the height of the support has been properly adjusted a $\frac{2}{3}''$ objective may be used. When ripe spores are being produced the slide is lifted out and placed for a few hours in a similar dish containing a little formalin, or a solution of osmic acid, in order to kill the fungus and partially fix the structures. The slide may be examined dry as it is, or, if the growth is too decidedly aerial, with a cover-glass laid on very gently. It is often possible, in this way, to make fairly flat preparations without breaking down fragile structures unduly. With some species permanent mounts may be made in lactophenol, afterwards cutting away the agar round the cover-glass and sealing with cement (see under Microscopical Methods).

The second way of making slide cultures is described by Henrici (1930, p. 33) and utilizes a shallow cell, built up on the slide with sealing-wax and a large rectangular cover-glass. Agar is run in to about half the depth—the plane of the slide being vertical—and the fungus is planted on the narrow surface thus provided. All stages of growth are readily observed owing to the spread of the mould being confined approximately to one plane and the effect is as if a thin section through a colony were being examined. With moulds of vigorous and coarse habit, such as many of the Mucoraceæ, a larger cell may be built up on the same lines, using sheets of thin glass, such as old photographic plates, clamped together in pairs with separators made from narrow strips of cardboard. The photograph of *Thamnidium elegans*, Fig. 21, was taken from a culture made in this way.

Another method of examining moulds *in situ*, in some ways more satisfactory than slide cultures, is to make cultures on "Cellophane" or other make of transparent viscose film. Cellulose acetate film is not suitable for culture work as it is non-absorbent. Since cellulose itself is utilized by comparatively few moulds, it is necessary to add suitable nutrients. There are two ways of doing this.

(1) Small pieces of the material (about $\frac{1}{4}''$ square is a convenient size) are soaked in dilute culture medium, blotted to remove excess liquid, and sterilized by steaming in any suitable receptacle. Using sterile forceps a number of pieces, six to eight are usually sufficient, are laid in a sterile Petri dish, or, if incubation is to extend more than a few days, in a dish lined with wet blotting-paper. Each piece is then inoculated at one or more points as desired and the dishes incubated.

(2) In the method described by Fleming and Smith (1944) small pieces of film are immersed in water in a short test-tube and sterilized by boiling or autoclaving. A number of pieces are picked out by means of sterile forceps, laid on the surface of an agar culture medium in a Petri dish and inoculated at one or more points. Soluble food material diffuses through the film and normal growth occurs. For the study of spore germination almost any culture medium may be used, but for examination of later stages of growth and spore production, it is better to use a medium of 10 per cent or even 1 per cent the usual strength so as to obtain a thin, transparent colony.

With either method single pieces may be removed at intervals for examination of various stages of growth, and may be handled in a number of different ways. They may be examined without any preliminary treatment, being simply laid on a slide or first bent sharply across the centre of the mould colony so that fruiting organs can be examined where these overhang at the fold. They can be mounted directly in lactophenol, or they may be first fixed in formalin vapour and stained with any reagent which does not stain cellulose. For permanent preparations they may be fixed, stained, dehydrated, cleared and mounted in balsam, the methods being fully described in a number of books on microtechnique. Two recent books which can be recommended are by Johansen (1940) and Sass (1941).

A further use of viscose film, described by Fleming and Smith (*loc. cit.*), is for the preparation of museum specimens of mould colonies. Circles of film (or of black or white paper which is not too highly glazed) are cut so that they just fit comfortably in a Petri dish. They are placed between layers of wet filter-paper in a dish and sterilized in the autoclave. Each piece is carefully laid on the surface of a culture medium in a Petri dish and inoculated at its centre. When a colony has grown sufficiently the film or paper is lifted out of the dish, floated on 10 per cent formalin and left overnight to kill and fix the colony, dried in the air and finally mounted between a pair of watch-glasses. Various modifications of the procedure are described in the original paper.

Cultures for Biochemical Studies. A number of methods have from time to time been used for growing moulds on comparatively large volumes of liquid media, for studies of biochemical activity and for isolation of metabolic products. The widespread interest in such studies at the present time, and the difficulties which have been encountered in some laboratories, are the reasons fo describing here in some detail the methods which have been foun satisfactory in the Department of Biochemistry at the Londo School of Hygiene and Tropical Medicine.

Containers. It is necessary, if moulds are grown as surface felts, t use shallow layers of liquid, in order to secure complete utilizatio of all the nutrients. Trays take up little room but are difficult t handle and to protect from contamination. Conical flasks, althoug taking up more space in the incubator, have been found to be i every way more satisfactory for total volumes up to 50 or eve 100 litres. Cultures can be examined, during the period of incubation, for uniformity, purity and vigour, distribution in a numbe of small containers localizes chance contamination, and the cours of metabolism is more satisfactorily followed by examination of the whole contents of a single flask than by drawing off samples from a large container.

Flasks are of good-quality resistance glass and are used solely for this kind of work. For isolation of products 1-litre flasks are used, but smaller sizes, from 100 ml. to 750 ml., are used for special purposes and for pilot experiments. The volume of liquid in each flask is such as will give a depth of not more than 4 cm. A normal 1-litre flask holds 350–400 ml., whilst a 250-ml. flask usually contains 100 ml.

Inoculation. Flasks are sown with an aqueous spore suspension (or suspension of mycelium if the fungus is sterile), giving a heavy sowing so as to ensure the rapid establishment of a continuous felt of mycelium. The most satisfactory method is to start with a number of cultures on agar slopes, in $\frac{3}{4}''$ or $\frac{7}{8}''$ rimmed tubes, allowing one slope for each 4 or 5 flasks if the mould spores well, and one slope for 3 or even 2 flasks if spore production is scanty or lacking. A similar number of tubes of sterile water, 12–15 ml. per tube, are also prepared. The contents of one tube of water are poured into one of the culture tubes, with, of course, the usual precautions, the surface of the agar is lightly scraped with a stiff tool, working as rapidly as possible, and the suspension is poured directly into the flasks. The aim should be to distribute the spores evenly, and not necessarily to add the same amount of fluid to each flask. There is usually no need to flame the mouths of the flasks, since the plugs are out for only a few seconds. Finally the flasks are shaken to distribute the spores. The whole process is carried through as rapidly as possible, consistent with efficiency, so as to avoid contamination. Rapid work also ensures that most of the spores are not completely wetted, and therefore float on the surface of the medium in the flasks.

A non-sporing mould is not so easy to handle as a species which spores freely. Flasks must then be sown with a suspension of bits of mycelium, which has the disadvantage that most of the inoculum usually sinks, and the formation of a surface felt does not begin until hyphæ from the submerged mycelium have extended sufficiently to reach the air, often a matter of several days. A method adopted with fungi which will not grow when submerged is to use a long needle to transfer bits of dry mycelium from a slope to the surface of the liquid medium, but to do this successfully requires practice.

In some laboratories adaptations of bacteriological technique are used. Cultures are grown in Roux bottles or flat culture flasks, spore suspensions are then made by scraping off the growth into comparatively large volumes of liquid, and flasks are sown with measured volumes of the suspension delivered from a pipette. This procedure does not usually give such satisfactory results as the method outlined above. Sowing with a pipette certainly means that each flask receives the same volume of suspension but seldom ensures equal distribution of spores, owing to the difficulty with which the spores of most species are wetted. There is also a much greater risk of contamination when many flasks are sown from one suspension than when only a few flasks are sown from one culture.

Special Media. Most of the culture media used for moulds are slightly acid in reaction, the *p*H being usually between 4·0 and 5·0. If a higher initial *p*H is desired, it is necessary to make up and sterilize the medium at a low *p*H and adjust to the final reaction after sterilization, in order to avoid decomposition of the sugar. The contents of one flask are used for a titration, to determine the requisite amount of alkali to be added. This amount of sterile alkali, usually NaOH, is added to each flask by means of a sterile pipette. It is advisable to have an assistant for this task, to flame the necks of the flasks and hold them in an inclined position whilst the alkali is being run in.

Media containing both glucose and ammonium salts cannot be sterilized without serious discoloration if the *p*H is higher than about 4·0. In such cases it is usual to dissolve the ammonium salt separately in a volume which is equivalent to an easily measured volume per flask, say 10 ml., and to dissolve the remaining ingredients in a correspondingly reduced volume. Both solutions ar sterilized and, when cold, the solution of ammonium salts is measure into the flasks by means of a sterile pipette. An easier way, an one sufficiently accurate for most purposes, is to pipette the solutio of ammonium salt, before sterilization, into a series of plugged tubes Then, after sterilization, the contents of one tube are poured into each flask.

Methods of Isolation and Purification of Moulds

The method used to isolate a particular mould from a natural substratum, and to obtain a pure culture, depends somewhat on circumstances. If the fungus is growing more or less luxuriantly, and typical aerial fruiting organs can be clearly seen, it is usually easy, working with a fine, sterile needle and with the aid of a good hand lens or dissecting microscope, to pick off a few spores, or a single spore head, and transfer to a suitable culture medium. Very often a pure culture results from this first transfer. It is seldom, however, that a mould is found growing, under natural conditions, entirely free from other organisms and there is always a danger that direct transfers will carry a contaminant. When this happens to be a slow-growing species its presence may not be detected for some time and, therefore, culture made in this way must be watched carefully over a period of several weeks, and purified if at any time there is reason to suspect contamination.

In many cases of mould growth on industrial products, such as leather goods, textiles and cereals, the presence of the fungus is betrayed only by a stain or discoloration instead of the more familiar furry growth. Even when the stain is due entirely to the presence of coloured spores, it is difficult, and often impossible, to demonstrate the presence of spore-bearing heads. In such cases direct cultures can seldom be made without introducing gross contamination. A few adventitious spores of a very rapidly growing species, in presence of a much larger number of those of the causal organism, may result in cultures being completely overgrown with a mould which has nothing whatever to do with the damage. With material of this type the best way of isolating the desired organism is by the operation known as "plating out."

Method. A few tubes of agar medium, 10–12 c.c. in each tube, are heated till the agar is melted and then placed in a water-bath maintained at 40–45°C. until required. In the meantime, a small portion of the mouldy material is reduced to as fine a state of subdivision as possible. A few fragments are dropped, by means of sterile forceps, into one of the tubes of medium, taking the usual precautions in handling the tube, the plug is reinserted and the tube is rotated between the palms of the hands to mix up the contents. The tube should not be shaken in the usual way as this introduces numerous air bubbles which are very persistent in the viscous fluid. The plug is removed, the mouth of the tube flamed and the contents poured into a sterile Petri dish. The medium from another tube is now poured into the first, mixed by rotation with the small amount of agar remaining after pouring the plate, and then poured into a second Petri dish. The contents of a third tube are now poured into the same tube, mixed and poured as before, and this process is continued for a number of plates which can only be determined by experience with the particular material; usually five or six plates are sufficient. As soon as the agar has set the plates are inverted and incubated.

The rationale of the process is simple. Thorough mixing of the infected material with the agar in the first tube serves to disseminate the spores of the fungus, or, in some cases, fragments of mycelium, throughout the melted medium. The small amount of agar left in the tube after pouring contains relatively few spores and these are again distributed throughout a considerable bulk of agar, and so on. Each plate, after the first, will contain only a fraction of the number of spores contained in the previous one and, on incubation, the successive plates will show fewer and fewer colonies. If the spores of a particular mould are very numerous in the infected material they are likely to persist through all the dilutions and give rise to colonies in the later plates, whereas a few purely adventitious spores, which are to be found on almost any material, are eliminated in the first two or three dilutions. The final plate, if the amount of material and the number of plates have been judged correctly, should show not more than nine or ten well-separated colonies at the outside. If these are all alike, a pure culture has been automatically obtained and nothing is required but to make transfers to slopes or fresh dishes as desired. If more than one mould is present, incubation of the plates should be continued only sufficiently long for the colonies to be differentiated, and transfers should be made immediately from any species which it is desired to study or retain. It is advisable to plate out separately each of the moulds isolated, as soon as the transfers are showing spores, in order to check their purity and effect further purification if this is necessary. It is policy, in a great many cases, to plate out on two or three different media, for an unimportant mould may grow so well on a rich medium, such as wort, that it swamps a slow-growing species, or, on the other hand, an important species may grow very poorly or not at all on a medium such as Czapek agar and be completely missed unless a more suitable medium is used as well.

Note. A slightly different method of making a series of dilution cultures is often advocated. Instead of pouring all the plates from one tube, which is refilled for each plate, a little of the thoroughly mixed contents of the first tube is poured into the second tube and the remainder poured into a dish, a little of the second is poured into the third tube, and so on. The method given above is better in two ways, unless an exact dilution ratio is necessary. It ensures an approximately uniform degree of dilution at each stage and each plate receives the same amount of agar, whereas, with the alternative method, the first plate receives too little and the last plate too much agar.

For the purification of an impure culture it is possible, if the contaminant is of slow growth compared with the chief species, to use the hyphal tip method. A single colony is planted in a Petri dish and, when it is about 1 cm. in diameter, a small piece of agar, containing the tip of a primary radiating hypha, is cut out and transferred to fresh medium. Another method is to make a transfer from a single spore head, using a fine needle and working under a good hand lens. The most usual method, however, is by plate dilution cultures, made as described above. It is necessary to use a very small amount of spore material to mix with the first tube of agar and, in cases where the spores are small and happen to be easily wetted, it is advisable to make one or two preliminary dilutions in tubes of sterile water or saline. Many workers prefer to make cultures which are obtained by germination of a single spore, this being an absolute guarantee of purity. It is extremely probable that the colonies on the last of a series of dilution plates are derived from single spores, but it is not absolutely certain, and it is therefore necessary to use one of the special methods, of which there are quite a number.

(1) A series of dilution plates are prepared in the ordinary way, using a clear, filtered agar. The plates are incubated only for

sufficient time for the spores to put out the primary germ-tubes and must, therefore, be examined under the microscope at frequent intervals during the first forty-eight hours. A few spores are found which are just germinated and which are well separated from all other spores. These are marked, whilst the dish is on the stage of the microscope, the agar cut round them with a sterile scalpel or the flattened end of a needle and the tiny pieces of agar transferred to fresh culture medium. The great disadvantage of this method is that the spores do not all lie in one plane and examination of the plates is extremely tedious.

(2) A better method is to flood the surface of the agar in a Petri dish with a dilute spore suspension in sterile water, allow to stand for a few minutes and then pour off the water. It will be found that quite a number of spores have stuck to the agar and, as these lie approximately in one plane, they are fairly easily found. Otherwise the procedure is the same as in (1).

A very convenient tool for cutting out minute blocks of agar enclosing single spores consists of a dummy microscope objective in which the front lens is replaced by a sharp-edged metal tube, about 5 mm. long and 1·5 mm. in diameter (this being approximately the diameter of the field using a $\frac{2}{3}''$ objective and a × 10 eyepiece). This is screwed on to the nosepiece of the microscope in place of one of the objectives, and, if possible, should be fairly accurately centred with the $\frac{2}{3}''$. The plate is examined with the latter lens and a field is found which shows a single spore. The cutter is swung into position and lowered until it touches the glass at the bottom of the dish. A small circular block is cut out of the agar but is left behind when the cutter is raised and may be re-examined with the $\frac{2}{3}''$ to ensure that all is well. If the cutter is not perfectly centred with the objective it is only necessary to make a few trial cuts, in order to determine the direction of the error, and thereafter to move the dish the determined amount before making the cut.

(3) A dilute spore suspension is made by shaking vigorously a small amount of spore material in a tube of sterile water or saline. A series of dilutions, in water or saline, are made from this until a suspension is obtained in such dilution that single loopfuls contain usually one, otherwise no spores. This is determined by spreading a series of loopfuls on a slide and examining under the microscope. Single loopfuls are then transferred to agar plates and the presence of a single spore in each is confirmed by direct examination. It is advisable to mark the positions of the drops on the bottoms of

the dishes so that the spores may be readily located after the drops have evaporated, and so that accidental infection may be recognized by its position on the plate. The plates are incubated and in those in which the spores are viable pure cultures are obtained.

(4) A very ingenious method is described by Hansen (1926) which works well with species which have large, coloured spores, such as species of *Alternaria*, *Helminthosporium*, etc., but is difficult to apply to such moulds as *Penicillia* and *Aspergilli.* A dilute spore suspension is made in melted agar medium. This is sucked up into a number of fine glass capillaries, of bore slightly greater than the diameter of the spores, and the medium is allowed to set therein. Microscopic examination of the capillaries should show short lengths containing each a single spore. These are broken off, sterilized externally with alcohol, and planted in fresh medium, when the spores germinate and the germ-tubes emerge from the ends of the capillaries and form typical colonies.

(5) A number of types of micro-manipulator are marketed with which it is possible to pick up a single spore and transfer it to a new substrate, all under a high power of the microscope. Unfortunately these instruments are very expensive and are not worth purchasing unless much more elaborate work, such as cell dissection, is to be attempted.

An excellent review of methods of single-spore isolation is given by Hildebrand (1938). The paper contains a detailed description of the author's technique and there is an extensive bibliography.

MICROSCOPIC EQUIPMENT

A microscope is absolutely essential for any work on micro-fungi. The determination of species involves the use of the highest powers and, whilst much good work has been and may be done by skilful manipulation of indifferent equipment, a really good outfit makes for ease, speed and accuracy. The microscope stand is largely a matter of personal opinion and of funds available, the products of any of the well-known manufacturers being entirely reliable. A focusing and centring substage is necessary, and a mechanical stage is desirable, for any serious work; even if an inexpensive stand is purchased, it should be seen that the former is fitted and that a mechanical stage and other refinements can be added as required.

The optical equipment should include a low-power objective, $1\frac{1}{2}''$ or $2''$, of short working distance, so that it can be focused on the surface of a culture tube laid across the stage without lifting the coarse adjustment out of its bearings, a first-class 8-mm. apochromat and a good oil-immersion objective of 2 or 3 mm. focal length. An ordinary $\frac{2}{3}''$ objective is also useful but not essential. The equipment which is always advertised as standard, $\frac{2}{3}''$, $\frac{1}{6}''$ and $\frac{1}{12}''$ objectives, is not a good investment for anyone contemplating serious work. The 8-mm. lens possesses certain very great advantages over the 4-mm. or $\frac{1}{6}''$. It has a larger field, it is comparatively insensitive to variations in cover-glass thickness, it can, with the normal length of drawtube, be adjusted to work on uncovered objects whereas the $\frac{1}{6}''$ cannot unless provided with a correction collar of extra wide range of adjustment, and, at equal numerical aperture, it has twice the depth of focus of the $\frac{1}{6}''$. In the same way the 3-mm. oil immersion has 50 per cent greater depth of focus than the 2 mm. when eyepieced up to give the same magnification and is therefore to be preferred for work on fungi, as we are usually dealing, not with thin uniform sections, but with comparatively thick, whole structures.

As regards eyepieces, the common Huyghenian form is suitable for use with low powers but does not work satisfactorily with apochromats or with high-power achromats. Compensating oculars are made for use with the latter, but there are several special types which are compensated for use with apochromats and have distinct advantages over the old compensating eyepieces in giving a larger and flatter field. Suitable powers are × 8 and × 12; in addition a × 20 ocular can be used to advantage with the 8-mm. apo. A measuring ocular is also essential and, since very great accuracy of particular measurements is seldom required, in view of the considerable normal variations in the dimensions of parts of a fungus, a glass disc micrometer, fitted in an ordinary eyepiece, is quite satisfactory. Under suitable conditions considerable accuracy can be attained with this simple and comparatively inexpensive apparatus, and it is far easier and quicker to use than the more elaborate filar micrometer. As far as the writer is aware, only one make of eyepiece, the "Periscopic" × 15, is so constructed that a disc micrometer resting on the diaphragm can be calibrated to give values of one division exactly equal to 1μ with the 2-mm. objective, 5μ with the 8-mm. and 10μ with a $\frac{2}{3}''$. With most oculars the best round figures which can be obtained are, respectively, 1·5μ, 7μ and 15μ. In the opinion of most microscopists it is preferable, except in very exact work which demands a more accurate type of measuring ocular, to calibrate micrometers at tube lengths which give round figures and to suffer the slight deterioration from perfection of image due to working at a wrong tube length, rather than be obliged to reduce all measurements to normal units by calculation.

As an accessory to a series of good objectives an efficient substage condenser is essential, if reliance is to be placed on observation of fine detail. The Abbe type is commonly used and is easy to manipulate, but a properly corrected achromatic condenser should be selected in preference whenever possible. Most English manufacturers make two forms, of which the one of larger diameter and longer focal length is somewhat easier to use and the better suited to low-power objectives.

No serious work can be done with daylight as an illuminant for the microscope. The ideal source of light is of small area and high and uniform intensity and undoubtedly the best available is the enclosed tungsten arc, typified by the "Pointolite" and "Pointlight" lamps. In order to obtain an image of the light source of reasonable dimensions, for use with objectives of lower power than 2 mm., it is necessary to use a bull's-eye condenser. As pointed out by Coles, a cheap and really efficient bull's-eye is the field lens (the larger of the two lenses) from × 6 Huyghenian eyepiece. It is not difficult to mount this on the lamp, with flat side towards the source of light and at a distance therefrom equal to the focal length of the lens. It should, if possible, be arranged to swing in and out from the optical axis so that it is always in perfect alignment when in use. Another suitable electric lamp, of lower intensity than the tungsten arc, is that described by Barnard and Welch. It utilizes an ordinary incandescent lamp and a stout glass rod, polished at one end and finely ground at the other, as a condenser. The multiple reflections inside the glass rod even out the inequalities in the beam of light and, when the rod is properly centred and focused, the ground end acts as a light source of almost uniform intensity. Where electric current is not available a very convenient gas lamp is the "Thorium Lamp," made by Messrs. James Swift and Sons, in which a small, circular pastille of thorium oxide is made incandescent by means of a flat, non-luminous flame. In spite of the convenience of electricity and gas, many workers still prefer the old-fashioned flat-wick oil lamp. With the flame turned edge on to the microscope it gives a very uniform light and one with which it is pleasant to work.

Whatever equipment the mycologist possesses it is essential, if accurate and reliable information is to be obtained by its use, that the worker should thoroughly master the technique of the instrument. Anyone who is new to microscopical work ought to read and digest one or two of the books recommended.

In addition to the apparatus already described, a good hand lens is very useful, particularly for the preliminary examination of cultures and observations of fungi growing on natural substrata. The best kind is the type known as "Aplanatic." It consists of three lenses cemented together and, although comparatively expensive, is worth the difference in price, compared with the singlets and doublets, in that it gives a much flatter field and is almost entirely free from chromatic aberration.

MICROSCOPICAL METHODS

When examining cultures for diagnostic purposes a great deal of information can be obtained by the study of dry, living cultures under the compound microscope. Slopes can be placed across the stage and the edges examined by transmitted light with objectives of $\frac{2}{3}''$ and lower power. Petri dishes can be laid flat on the stage, either side up, and examined by incident or transmitted light. If the mould forms dense, matted growth, the planting of several colonies in one dish will often result in narrow sterile zones, where the colonies approach each other, and mature fruiting organs can be observed partially overhanging these clear spaces. In the case of *Aspergilli* and *Penicillia* the shape of the fruiting heads, the disposition of the chains of spores and the origin of the conidiophores, whether from submerged or aerial hyphæ, have considerable diagnostic value, and the required information can be obtained only from the study of undisturbed, living cultures. In the same way, determination of species of *Mucor* and *Rhizopus* is possible only when study of slide preparations is combined with examination of living material in Petri dishes. Many other moulds have conidial structures, which fall to pieces at the least touch and, with these, the value of direct observation is apparent.

When examining living cultures it is important to remember that a good deal of water is given off by fast-growing species, and some of it appears on the hyphæ as small droplets, invisible to the naked eye. It is not uncommon to see the sporangiophores of species of *Mucor* thickly studded with these droplets, and by the beginner they may be readily mistaken for chlamydospores. In test-tube cultures an appreciable amount of water often collects on the wall of the tube. Sporangia coming in contact with this usually burs and liberate an irregular mass of spores. Similarly, false spore heads held together by mucus, such as those of *Verticillium* and *Cephalosporium*, are broken up. Spores often germinate in such dam situations, even whilst still attached to sterigmata or in sporangia, and give rise to structures which are puzzling the first time they are seen under a low power of the microscope.

For study of fine detail, and for accurate measurements, slide preparations must be made. With most species of moulds it is difficult, if not impossible, to fix, stain and mount specimens, as is done for botanical and zoological material, and at the same time preserve structure. In all but a few cases it is general practice to use fluid mounts prepared with as little manipulation as possible. Water is seldom suitable as a mounting medium. It evaporates rapidly, it causes shrinkage of hyphæ by osmosis and it usually causes the parts of the specimen to adhere together as a tangled mass of hyphæ, spores and air bubbles. Alcohol wets efficiently and makes a fairly satisfactory mountant for a brief and rapid examination, but is too volatile for making permanent slides. Easily the most generally useful medium is that known as "lactophenol."

A number of formulæ for this medium have been published but, for general use, the original recipe of Amann (1896) is to be preferred.

Lactophenol

Phenol (pure crystals)	10 g.
Lactic acid (syrup, sp. gr. 1·21)	10 g.
Glycerol (pure)	20 g.
Distilled water	10 g.

It is readily prepared by warming the phenol with the water until dissolved and then adding the lactic acid and glycerine. The refractive index is 1·45.

The method of mounting is to place a *small* drop of mounting fluid in the centre of a clean glass slide, with a sterile needle pick off from the culture a very small portion of typical material, place this in the drop of fluid and very gently tease it out with a pair of needles until it is well wetted, then lower on to it a cover-glass in such a way as to avoid air bubbles as far as possible. It is difficult to make slides which contain no air bubbles without teasing out the specimen to such an extent that structure is destroyed, and within reason, their presence does not seriously interfere with observation. Some moulds are very difficult to wet and, in these cases, the slide, before the cover-glass is put on, may be gently warmed over a small flame in order to expel most of the air. Another method of expelling air, which has been found particularly useful for the biverticillate *Penicillia*, is to tease out the specimen in alcohol, then displace this with two or three successive large drops of lactophenol, taking up most of the fluid on filter paper before each subsequent addition. If even this fails to give a presentable slide, as may happen with thick structures such as perithecia, the specimen should be placed in a small watch-glass, covered completely with mounting fluid and the whole placed in any vessel from which the air can be exhausted. A vacuum desiccator, if available, will take several specimens at once and is admirable for the purpose. To make sure of the removal of all air from the specimen, air is admitted to the vessel after 10–15 minutes and then suction is again applied for about another 10 minutes. The specimen may now be transferred to a slide and a cover-glass applied. It is usually impossible to make good slides by using mounting fluid in such quantity that it just fills the space below the cover-glass (this is the practice when Canada balsam is used as mounting medium). Generally an appreciable amount oozes out, especially if the cover-glass is gently pressed down with a pair of forceps, and must be absorbed on bits of filter paper carefully applied to the edge.

The advantages of lactophenol are that it rarely causes either shrinkage or swelling of the cells of most fungi, and that it has no tendency to evaporate, so that preparations are reasonably permanent, if carefully handled, without any further manipulation. It is also sufficiently viscous to allow the use of an oil-immersion lens without undue movement of parts of the specimen, provided that the immersion oil has not become oxidized. The main disadvantage is that the refractive index of the medium differs little from that of fungal hyphæ, with the result that unstained and colourless structures are not readily visible. In many cases, therefore, it is advantageous to stain the specimen.

In general, moulds tend to take stains somewhat unevenly, due to the fact that their affinity for stains, which in young structures is considerable, decreases rapidly with age. The simplest method of staining is to mix the stain with the mounting fluid, but only a few dyes can be used successfully in lactophenol, the chief being cotton blue, picric acid and picro-nigrosin. Cotton blue (0·05 g. in 100 ml. lactophenol) stains young structures fairly deeply but with most specimens acts very unevenly, a single hypha often showi irregular patches of colour. The picric acid medium is made substituting a saturated solution of the dye for water when co pounding lactophenol. It stains evenly, compared with cotton blu but the colour is rarely sufficiently intense. Contrast may be i proved, especially for photography, by using a blue light filter the microscope lamp. With both these combined staining a mounting fluids most of the stain is absorbed by the specime leaving the "background" almost colourless. Picro-nigrosin is ma by dissolving 2 per cent of water-soluble nigrosin (bacteriologic stain) in saturated aqueous picric acid and using this solution i place of water when making up lactophenol. The concentration nigrosin in the mounting fluid is 0·4 per cent. Nigrosin alone, i aqueous solution, does not stain fungi and is actually used for th so-called relief or negative staining, in which the fungus is mounte in a 5 or 10 per cent solution of nigrosin and appears as a colourles image on a blue-black background. In combination with picric aci it stains rapidly from lactophenol solution, the colour produce being a neutral grey. Mounting directly in this stain medium usuall gives too much background colour and it is best to allow the stai to act for about half a minute, without teasing out the specimen suck off as much as possible with bits of filter paper, and finall mount in plain lactophenol.

A method which is often satisfactory and which, with care, gives even staining with adequate contrast, is to treat the specimen first with a solution of the stain in acetic acid or alcohol and then replace this fluid by lactophenol. Alcorn and Yeager (1937) recommend Orseillin BB, used as a 0·25 per cent solution in 3 per cent acetic acid. The author has obtained good results with Orange G, using a 1 per cent solution in 3 per cent acetic acid. In either case the specimen is immersed in a drop of the stain, on a slide, and allowed to remain for 3–5 minutes. It is then examined under the microscope and, if too deeply coloured, is treated with successive drops of very dilute acetic acid until satisfactory. The stain is then displaced by several successive drops of plain lactophenol and the slide finished in the usual way. Another stain which works well with young structures and, incidentally, shows up the nuclei, is Chlorazol Black E (E. Yuill; private communication). The stain is used in 1 per cent solution in alcohol (90–95 per cent) and is allowed to act for about one minute, using alcohol to differentiate if staining has been too rapid.

In the case of all slides which are intended to form a permanent record, the edge of the cover-glass should be sealed with a suitable cement. Otherwise, it is very difficult to clean the cover of an old slide without seriously disturbing the specimen. Unfortunately there is at present no completely satisfactory cement for use with lactophenol mounts. Brown shellac cement is, on occasion, admirable, but it is not wholly dependable, as sometimes, even when every care is taken, it tends to leak under the edge of the cover-glass and form unsightly bubbles and patches. The following are the main conditions for success: (1) The cement must be of suitable consistency. It should run smoothly but not too readily from a brush. (2) No cement should be applied until it is certain that the cover-glass is not under strain. If a slide is allowed to stand for a few days, it is often the case that air bubbles appear at the edge, showing that the cover-glass has been bent slightly when making the slide and has gradually recovered on standing. Such air spaces must be filled in with mounting fluid before applying cement. (3) Using a turntable, a very thin coat of cement should be put on first with a fine brush. When this is quite dry, a second thicker coat is added to overlap the first. Another cement, which has no tendency to leak under the cover-glass but which is somewhat messy to use, is made by melting together four parts of resin and one part of lanoline. It is applied hot to a previously warmed slide. Some workers use square cover-glasses and apply the cement free-hand, rather than attempt to put it on with brush and turntable.

A different method of sealing was described originally by Diehl (1929). The object is placed in a drop of mounting fluid on a 22-mm. No. 0 cover-glass and is then covered with a 12-mm. No. 2 cover-glass. Any excess fluid is carefully cleaned away from the edge of the small cover, a large drop of Canada balsam is placed in the centre and a slide is gently lowered on to it. When the balsam has spread to the edge of the large cover-glass the slide is inverted and allowed to stand till the balsam has set. Linder (1929) claims that this method can be used with lactophenol provided that the mount is dehydrated in a desiccator before the final mounting in balsam. However, most workers who have experimented with this method have found that lactophenol and balsam are not compatible, the balsam gradually creeping between the cover-glasses and eventually ruining the slide. It is to be hoped that a more suitable cement will be discovered since, in theory, this is an ideal method of mounting.

It is unfortunately true that some slides deteriorate rapidly
spite of every care in preparation. As stated above, spoilage ma
result from the leakage of an alcoholic cement under the edge
the cover-glass, but there is a more common cause of trouble whi
is entirely unconnected with the method of sealing. The mycel
of many species of fungi contain large amounts of fat and there
a tendency for this to be displaced by mounting fluid, with t
consequent appearance of numerous unsightly globules all over t
slide. In such cases it is not possible to prepare satisfacto
permanent slides by this simple technique.

Whatever the method of preparation, all slides which are to
kept should be adequately labelled as soon as made, giving the nam
of the species, the stain (if any) and the date of preparation.

One further point about the use of lactophenol should be note
It is a non-swelling medium and, therefore, measurements of objec
in this fluid will not agree with measurements of the same objec
mounted in water, which often causes appreciable swelling of fung
hyphæ and spores. Many published diagnoses of fungi includ
measurements recorded from specimens mounted in water (althoug
there has been for some years an increasing tendency to use lact
phenol) and it is necessary to bear this in mind when trying t
identify moulds.

In the examination of slides beginners are often puzzled by certa
artefacts. The commonest perhaps are very small air bubble
These are usually circular if the layer of medium is thick, but ma
be of almost any shape if the cover-glass has been well presse
down. The circular ones have a very thick dark outline and loo
as they are, very refractive. The thin ones have dark edges, bu
the most noticeable point about them is that portions of hyphæ o
spores lying inside the bubbles are not in focus when the rest o
the slide is sharp, and also show a greater degree of contrast tha
the same structures surrounded by fluid. Another common appea
ance is a series of circular bodies of very varying size, somewha
refractile and always with smooth edges. These are oily substance
exuded from the cells of the fungus, or squeezed out during th
mounting process. As mentioned above, these oil drops ofte
increase in number when a slide is stored and may entirely ruin th
preparation. It is not uncommon, when making slides from ver
thin colonies on agar, to carry small bits of culture medium to th
mounting fluid. These become flattened out when the cover-glass
pressed down. The first thing noticed about such patches of aga

is that they do not take the stain from coloured lactophenol. They usually contain submerged hyphæ and often have a few spore heads pressed into them. The latter always appear somewhat shrivelled and distorted and are, like the agar, unstained.

IDENTIFICATION OF SPECIES

Methods of obtaining pure cultures and general methods of examination having been discussed, it now remains to describe the routine to be followed when it is desired to identify an unfamiliar species.

The first necessity is to prepare a full and accurate description of the mould, as grown on standard media. It is assumed that some idea of its general behaviour on one or two media has already been gained during isolation and purification. If the species grows satisfactorily on Czapek agar this should now be used, but if the Czapek medium gives poor and stunted growth, or if sporing structures are lacking or tardily produced, some other medium must be selected. Several cultures should be made in Petri dishes. One or two of the dishes may be planted with single colonies for measurement of growth rate, the rest with several colonies spaced about an inch apart. It is necessary to make a number of cultures because some of them will be examined, probably with the dishes uncovered, before maturity, and are likely to develop infections on continued incubation. Cultures should be examined at frequent intervals and the following details recorded:

1. Rate of growth; described as slow, very slow, moderate, rapid, etc.
2. Colony colour and colour changes; whether uniform or in zones or patchy. Evanescent colours which are often to be observed at the edges of growing colonies should be recorded.
3. Colour and colour changes of the reverse of the colony.
4. Colour changes in the medium; whether confined to the area covered by the colony or diffusing.
5. Texture of surface; whether loose or compact; plane, wrinkled or buckled; velvety, matted, floccose, hairy, ropy, gelatinous, leathery, etc.
6. Odour, if any.
7. Character of drops of transpired fluid often found on aerial growth.
8. Character of the submerged hyphæ; colour, presence or absence

of septation, approximate diameter, characters of special structure if any present.

9. The stage at which fruiting structures develop.

10. The character and disposition of the mature fruiting organs whether sporangia, perithecia, pycnidia, stromata, sporodochia coremia or detached conidiophores; whether borne in the substratum, on the surface or on the aerial growth. The presence of more than one type of sporing structure should be particularly noted.

11. Colour, size and shape of mature fruiting organs or fruit-bodies

12. Details of structure of the fruiting organs, including measurements of essential parts and disposition of the spores thereon.

13. Full details of spores; colour, shape, septation, surface markings, size (including both average and extreme measurements)

Data numbered 1–7 are obtained by examination of cultures with the naked eye or with a hand lens, 8–11 by the use of a low or moderate power of the microscope, observations being made on the living cultures. Numbers 12 and 13 necessitate the preparation of slides and the use of the highest powers of the microscope.

The information recorded under 8 and 10 should be sufficient to place the species in its correct class and order. For example, if the vegetative hyphæ are comparatively coarse and without septa, and sporangia are observed, the fungus must be placed in the Zygomycetes, and in the order Mucorales. Consideration of the rest of the data will lead to the family and then the genus. The determination of the actual species is, except in a few genera, a matter of some difficulty. If the genus has been monographed it is usually a question of patient and careful observation, along the lines indicated by the authority, then repeated consideration of the data until the unknown fits into its proper place. In absence of an authoritative treatment of the genus the usual procedure, and the quickest in the long run, is to consult Saccardo's Sylloge, look up all the recorded species in the genus and follow up references to any which seem to be near the one to be identified. Unfortunately, many species have been inadequately described and there are even numerous genera which are ill-defined. In some cases the accepted conception of a genus is a matter of tradition rather than adequate diagnosis, and it is difficult to find any published data sufficiently exact for recognition. However, the genera which are of commonest occurrence and greatest importance are just the ones which have been most studied and concerning which there is an extensive literature.

One thing the student should guard against. It is doing a great disservice to other mycologists to assume too hastily that an unrecognized fungus is a new species and to publish a description under a new name. The literature is cumbered with a mass of generic and specific names which are nothing more than synonyms of well-known forms, and which ought never to have been bestowed.

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For the new one-volume edition much elementary descriptive matt
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It includes methods of preparation of slides of all kinds of subjects

CHAPTER XI

PHYSIOLOGY OF MOULD FUNGI

THE growth of fungi, like that of all other living things, is profoundly influenced by environment. Variations in external conditions may not only affect rate of growth, but in many cases, can bring about differences in type of growth. To make a very broad generalization, fungi resemble the higher plants in that they produce vegetative growth, that is mycelium, when food is abundant and conditions are favourable for easy assimilation, and produce fruit-bodies in response to conditions which tend to check rapid growth. In addition, unfavourable environment may result in dwarf forms being produced or in the appearance of structural abnormalities. In some cases the difference between normal and abnormal growths of a single species is as great as that between different species or even different genera.

The various topics treated below are only discussed in broad outline and there has been no attempt to take account of all the great mass of published papers relating to the subject.

Food Requirements. Water and oxygen are necessary for growth of fungi and, in addition, the following elements are known to be required: carbon, nitrogen, potassium, phosphorus, sulphur and magnesium. In addition, many require, or grow very poorly in absence of, iron. Opinions vary as to the necessity of a few other elements but, if any are required, the amounts are so minute as to make special provision of such unnecessary in culture media. Most of the common moulds can utilize inorganic sources of all the elements except carbon, but a few are unable to use inorganic nitrogen. Others grow well on media containing ammonium salts but cannot utilize nitrogen from nitrates. However, the great variety of natural substrata which are commonly infected by numerous species of moulds indicates that these organisms can tolerate wide variations in concentrations of the essential elements and can utilize both carbon and nitrogen combined in very diverse forms.

In addition to essential elements fungi can utilize a few other elements whose presence or absence seems to have no effect on growth. Raulin analysed the ash of *Aspergillus niger* and, on the basis of his analysis, devised his well-known culture medium. One of the ingredients, potassium silicate, is certainly not essential, but, when it is used, silica is found in the ash of the mould. Chlorine is not necessary for growth and the potassium chloride often added to culture media (as in the Czapek-Dox solution) is used as a convenient source of potassium. A number of fungi can, however, utilize the chlorine contained in such media, building up complex organic compounds containing chlorine. Raistrick and Smith (1936) showed that one strain of *Aspergillus terreus* could utilize over 95 per cent of the chlorine contained in Czepak-Dox solution, most of it being found in two compounds, "geodin," $C_{17}H_{12}O_7Cl_2$, and "erdin," $C_{16}H_{10}O_7Cl_2$. Clutterbuck *et al.* (1940) have published a survey of the chlorine metabolism of a large number of species of moulds and shown that the majority can utilize this element to a small extent, whilst a few metabolize over 25 per cent of the total chlorine in the medium, an outstanding species being *Caldariomyces fumago* Woronichin (commonly known as *Fumago vagans* Pers.). This species utilizes about 95 per cent of the chlorine in the medium, producing a compound named "caldariomycin," $C_5H_8O_2Cl_2$. Two other chlorine-containing metabolic products of moulds have been described in recent years, "griseofulvin," $C_{17}H_{17}O_6Cl$, from *Penicillium griseofulvum* (Oxford, Raistrick and Simonart, 1939), and "sclerotiorine," to which the formula $C_{20}H_{20}O_5Cl$ is assigned, from *Pen. sclerotiorum* (Curtin and Reilly, 1940).

The actual quantity of food material required to support mould growth is very small. Fungi are frequently to be found growing on polished furniture woods, or even on old iron, where the total amount of available food must be exceedingly minute; scientific workers in the Tropics have often recorded that microscopes and other optical instruments have been rendered unusable by mould growth adhering firmly to, and often etching, the lenses and prisms; textiles made of cellulose—cotton, linen and artificial silks—may be extensively "mildewed" without the fabric showing the least sign of weakening or tendering, the only available food material being apparently the small amounts of substances other than cellulose which are contained in all such fabrics; solutions of many inorganic salts, on standing, often develop slimy growths of mould mycelium and it is common experience that many species can grow and produce spores on plain agar made up with tap-water, a medium which, since the agar is not utilized, is extremely poor in nutrient material.

Respiration. All fungi require oxygen for growth. Many of the yeasts develop characteristically when completely submerged in liquid, where the amount of available oxygen is small, and a few other fungi produce atypical structures, resembling the yeasts, when grown under similar conditions, but no fungus is truly anaerobic. The spores of a great many filamentous species will germinate when immersed in liquid media but grow very slowly until some of the hyphæ reach the surface, after which they spread rapidly and normally until the surface is covered.

Normal metabolism results in the breakdown of some of the organic food material to CO_2 and, unless this is continually removed and replaced by a fresh supply of air, growth ceases, whether on account of the diminution in oxygen tension alone or by actual poisoning is not known. In the laboratory, vessels in which fungi are grown are usually plugged with cotton-wool, and it might be supposed that this would restrict aeration. Moulds are commonly grown in liquid media in test-tubes 6″ long, the depth of liquid being about 1″, and it would be reasonable to assume that, with the tube in a vertical position, CO_2 would accumulate above the surface of the liquid. In a culture flask, with a much greater active surface relative to the plugged opening, conditions should be much worse, yet analyses of air drawn from flasks containing actively growing moulds invariably show that there must be a very rapid removal of CO_2 and intake of fresh air.

It is readily shown, by passing measured volumes of air through a culture vessel, or through a number of vessels in series, that lack of air influences rate of growth and, in many cases, alters the type of growth. One common effect of very restricted aeration is to suppress normal colour production and a very striking instance of this is mentioned by Thom in The Penicillia (1930, p. 83). He states that when Roquefort cheese is cut the fresh surface is often colourless but turns green very rapidly on exposure to air.

Reaction of Medium. Most of the commonly occurring moulds will tolerate a wide variation in hydrogen-ion concentration provided other conditions are favourable, but, as might be expected, different species respond in various ways to changes in reaction. In general, a slightly acid medium is favourable to spore germination and rapid growth of the young colony, just as a slight alkalinity is preferred by the majority of bacteria. Once growth is established the reaction of the medium usually changes owing to the accumulation of products of metabolism. Many species form fairly large quantities of organic acids, oxalic, citric and gluconic acids being very commonly found and many others more rarely. In absence of metabolic products of a strongly acid nature, alteration in reaction of the culture medium depends largely on the types of inorganic salts present. For example, the utilization of nitrogen from sodium nitrate tends to liberate base and increases the pH, from ammonium sulphate to liberate acid and decrease the pH, whilst the presence of appreciable amounts of buffer salts tends, of course, to stabilize the reaction. It is not unusual for appreciable quantities of organic acids to be produced during the early stages of growth and then to be utilized by the mould as other sources of carbon become exhausted. In such cases the pH of the medium will first decrease and then gradually increase as growth proceeds. Sometimes the appearance of the mould itself indicates changes in reaction of the medium. In many of the green species of *Aspergillus* and *Penicillium* the green colour of the young colony is very stable, often persisting for several months, on a heavily buffered, acid medium such as wort, whereas on Czapek's medium, which tends gradually to become alkaline unless strong acids are synthesized, the initial green slowly changes to brown or grey shades. Many mould pigments, produced either in the mycelium or in the medium, act as indicators, showing very distinct colour changes according to alterations in reaction.

Influence of Light. It is impossible to generalize concerning the effect of light on the growth of fungi, for whilst many common species seem to grow equally well and characteristically in light or in darkness, others are definitely stimulated or affected by light. For example, a culture of *Rhizopus nigricans*, growing in a Petri dish, was clamped on the microscope stage and the edge of the colony was observed with a 2″ objective and a low-power eyepiece fitted with a micrometer scale (magnification approximately 15 ×). In diffused daylight the extension of the radiating hyphæ could actually be seen and, after an hour and a half the edge of the colony had advanced almost half the diameter of the field. When the whole apparatus was placed in the dark the advance was less than half that observed in the light, showing that, in this species, there was a very definite stimulation of vegetative growth by comparatively weak light. Some fungi are positively phototropic; that is, they grow towards the light or their fruit-bodies turn in the direction of maximum illumination. The sporangiophores of *Phycomyces nitens* are short when a culture is fully exposed to light but attain a length of 20–30 cm. if the mould is grown on a layer of medium placed at the bottom of a tall vessel which allows light to enter only from the top. Fig. 139 shows a very characteristic growth of *Ph. nitens* in a

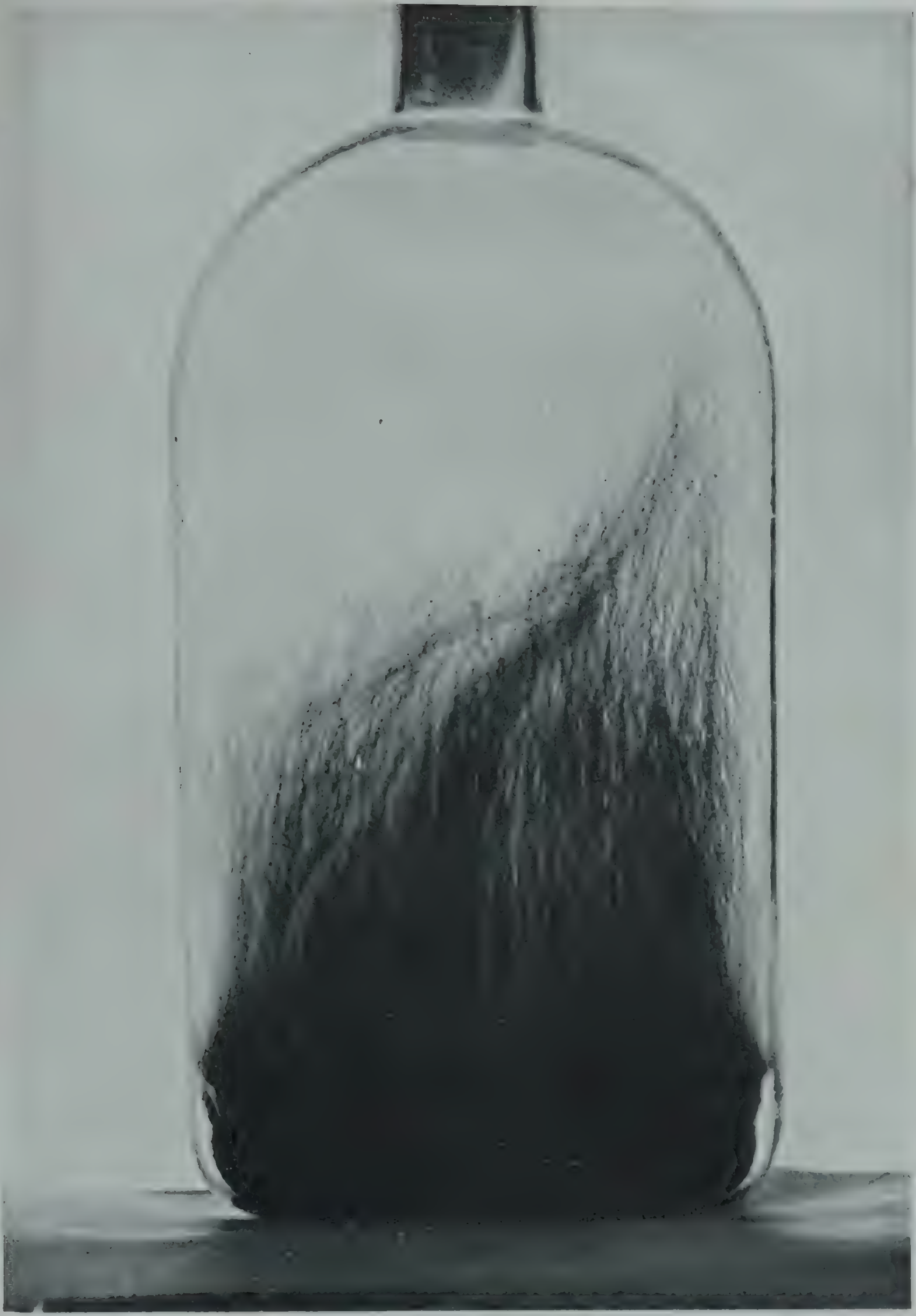

Fig. 139.—*Phycomyces nitens*—culture in Roux bottle, illuminated from one shoulder only during period of growth, showing well-marked phototropism. × 0·5.

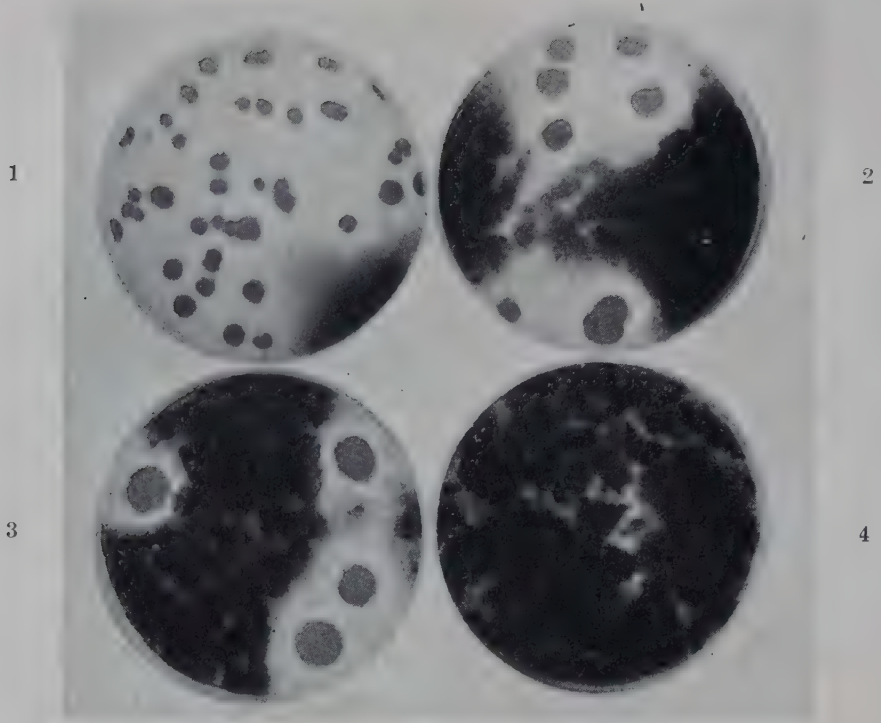

Fig. 140.—Four of a set of dilution plates containing colonies of *Penicillium* and *Stachybotrys*. × 0·35.

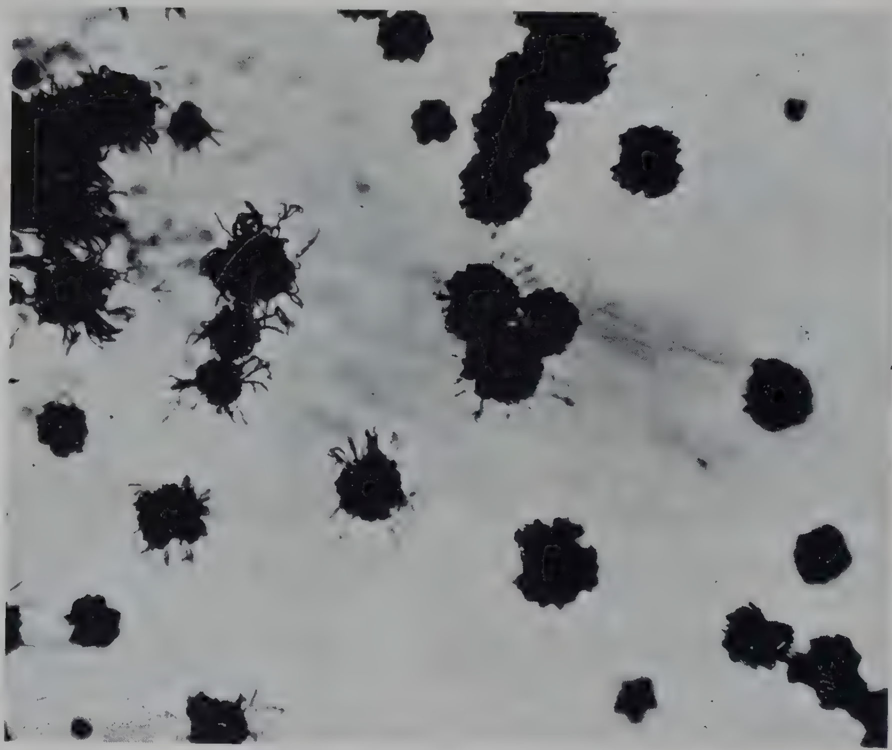

Fig. 141.—*Penicillium* disease of *Aspergillus niger*—infected heads as seen in Petri dish. × 25.

Roux bottle which was covered with opaque material except for a patch on one shoulder, the sporangiophores being all turned towards the point of entry of the light. A number of other species exhibit similar tendencies. The sporangia of *Pilobolus* species regularly incline in the direction of strongest illumination ; the stipe of the wood-destroying fungus, *Lentinus lepideus*, grows towards the light and the cap develops only when the intensity of the light exceeds a certain minimum; the necks of the perithecia in some of the Pyrenomycetes are regularly orientated towards the light ; and it is probable that, in all such cases, phototropism represents an adaptation for the better dispersal of spores.

The effect of light on some species is to stimulate the production of fruit-bodies, exposure to sunlight or ultra-violet rays being often a useful method of inducing sporing in cultures which have become purely mycelial. Pigment production is also quite frequently initiated or enhanced by the influence of strong light.

Temperature Relationships. Fungi show great differences in their response to temperature changes and in their powers of resistance to heat and cold. Application of heat is, of course, the usual method of sterilizing culture media, vessels and tools and of killing unwanted cultures, there being few moulds which can withstand the action of steam or boiling water for any appreciable time. Thermal death points, however, cover a wide range, some strains of *Penicillium brevi-compactum*, for example, being killed by prolonged exposure to a temperature of 33 C., whilst *Byssochlamys fulva*, a fungus responsible for much trouble in the canning industry, can survive the normal sterilization process in which, for a short time the temperature exceeds 90 (Olliver and Smith, 1933). On the whole, spores are more resistant than mycelium and both are less affected by dry than by moist heat. In the same way the spores of many species are killed by freezing in presence of water but when dry have their germinative powers unimpaired by prolonged cooling in liquid air.

The limiting temperatures which will admit of growth, as distinct from mere retention of vitality, are less extreme. Brooks and Hansford (1922), and Brooks and Kidd (1921) have shown that *Cladosporium herbarum* will grow, and its spores germinate, on meat in cold storage at a temperature of —6 C., and several other species will grow at slightly higher temperatures which are still below freezing-point. Numerous other workers have confirmed that many species can grow at or just below 0°C. At the other end of the scale, *Aspergillus fumigatus* is thermogenic as well as thermophilic an often found flourishing about 50°C., a temperature at which nea all other fungi are inhibited and many of them killed. Quit number of common moulds, including many *Mucors*, *Aspergilli* a *Penicillia*, grow well at 37°C., but many others are inhibited.

Each species is found to grow best at round about some particu temperature, known as the optimum temperature for that speci On the whole, species of *Penicillium* have optima between 20° a 25°C., whilst the nearly related *Aspergilli* grow best at about 3 This is a very rough generalization, with many exceptions, but often of value in industrial work, where 90 per cent of the fu encountered belong in these two genera. One particular section the *Aspergilli*, the *A. glaucus* group, deserves special mention, most of the strains have two optimum temperatures. Conidia a purely vegetative growth are produced most freely at somewh low temperatures and, therefore, temperature optima, as dete mined from growth rate, are low, for some strains as low as 1 Perithecia, on the other hand, are formed most readily and abu dantly at higher temperatures, some species being almost entire perithecial above 30°, and there is thus a second optimum relati to the perfect form of the fungus. Although the majority of t *Penicillia* have optima which are comparatively low, there are number of species, mostly belonging in one group, the Biverticillat Symmetrica, which show maximum growth at temperatures near t 30° and which flourish up to at least 37°.

Moisture Requirements. A supply of water is absolute essential for the growth of all fungi and, although a few speci can thrive in presence of very small amounts of available moistur it is fortunate that the great majority of moulds require what ma be termed damp conditions. Dry rot appears only on timber whic has not been thoroughly seasoned or which is exposed in a humi stagnant atmosphere, and mould appears on wall-paper only whe joints in the wall are faulty and the paper itself has become pe ceptibly damp. Many industrial products, paper, textiles, leathe and food-stuffs of various kinds, readily absorb or adsorb wat from a humid atmosphere and are liable to attack by but a ver limited number of moulds except when stored under conditio where this intake of water is excessive.

It is important to realize that the total amount of water containe in any particular substance does not necessarily determine i liability to fungal attack. Jam, for instance, contains a hig percentage of water but, if properly compounded, does not become mouldy because the high concentration of soluble material, chiefly sugar, renders the water unavailable to fungi, whereas a material consisting chiefly of cellulose, such as cotton or paper, is liable to attack if the moisture content exceeds about 8 per cent. Glycerine is often considered to have antiseptic properties but, at low concentration, it is an excellent food for many fungi and it inhibits growth only in concentrated solution, when the effect is chiefly or entirely due to its desiccating action and not to any specific toxicity. Of all the fungi which can tolerate high osmotic concentrations of dissolved substances easily the most important are members of the *Aspergillus glaucus* series. These are frequently encountered on all types of material which contain only very small amounts of excess moisture. They are commonly found, as pure cultures, on jams and similar products which contain rather less than the normal percentage of sugar. Even more tolerant of high concentrations of dissolved substances are the so-called osmophilic yeasts, which are commonly found as causes of spoilage of honey.

The question of moisture requirements is further discussed in Chapter XIII.

Poisons. The Fungi, like other classes of living things, are affected adversely by the presence of certain substances in their food supply. Our knowledge of what might be termed poisons has been gained chiefly through the demands of various industries for efficient antiseptics to combat the ravages of fungi amongst their raw materials and products. This topic is discussed further in the chapter on "Control of Fungi" (Chapter XIII) and will not be elaborated here.

It is an interesting fact that the use of certain poisonous substances, in very small doses, as general tonics for the human system is paralleled by the use of small quantities of antiseptics for stimulation of fungal growth. Zinc salts are probably the best known of such substances and are regularly incorporated in certain well-known culture media, but many other substances show the same reversal of effect on particular species or groups of species.

Influence of Other Fungi. If several species of fungi are growing together on the same substratum they may affect each other's growth in various ways. In the simplest case there is competition for the available food material and the ultimate result is that some species are prevented from spreading, or starved out of existence, whilst others flourish. It is impossible to predict, from knowledge of the individual species, which will be the survivor a mixed culture, since rapid growth in pure culture does not ne sarily mean ability to acquire food in face of competition. In m cases of mixed infections it is found that some of the moulds g abnormally, producing freak structures or becoming dwarfed, it is therefore very unsafe to attempt to base identifications examination of such material. Fig. 140 is a photograph of the f four of a set of dilution plates made from a mixed culture o *Stachybotrys sp.* and a *Penicillium sp.* The fact that the fourth pl still contains many colonies of the *Stachybotrys* (black) shows t the fruiting of this species has been completely suppressed on plate containing many *Penicillium* colonies (grey).

Some species, when growing in proximity to other moulds, p duce circular colonies surrounded by a clear zone in which the grow of the competing organisms is inhibited. Weindling (1932, 19 1936, 1937) has shown that *Trichoderma lignorum* and a gre *Gliocladium* show such an inhibitory effect to a marked degree, a has isolated the toxic substance. He has also shown that hea inoculation of *Trichoderma* into wet soils can favourably influen the growth of crops by suppressing damping-off fungi. A use review of the whole subject of competition amongst fungi has be published by Porter and Carter (1938), and the subject of antagoni between micro-organisms is further discussed in Chapter XIV.

A very common state of affairs, when a suitable substrate exposed to chance infection, is for there to occur a well-mark progressive change in the fungal population, probably correlat with the different enzymatic activities of the various types. O group breaks down complex organic material to provide suita food for another group, whilst a third group will utilize the met bolic products of the second. Many fungi, for example, are unal to attack cellulose but flourish luxuriantly on the products of partial decomposition. Amongst the coprophilous fungi there usually a well-marked sequence, the primary flora of dung consisti of Mucorales, followed by Ascomycetes, then by Basidiomycetes.

True parasitism amongst the fungi is by no means uncommo The higher fungi, particularly the large fleshy forms, are attack by a large variety of micro-fungi and even a few of the forn commonly grown in the laboratory are parasitized, usually nearly related species. Thus a number of Mucorales, most fr quently species of *Chætocladium* and *Piptocephalis*, attack oth members of the same order, and *Penicillium rugulosum* grows cultures of various *Aspergilli*, most commonly, *A. niger*, forming masses of olive-green fruits over the heads, and eventually killing them (see Fig. 141).

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CHAPTER XII

THE MAINTENANCE OF A CULTURE COLLECTION

In most laboratories where serious work on micro-fungi is carrie out it is necessary to maintain a collection of moulds. If the wor in hand aims at controlling the harmful activities of fungi on som industrial product most of the preliminary work will have to be dor on pure strains of moulds isolated from actual cases of damage, an every important species will have to be kept in pure culture so as t be available for numerous experiments extending, perhaps, over period of years. Those who study the biochemical activities c fungi, with a view to isolating or manufacturing special produc of metabolism, usually find it necessary to test many strains c the same or related species, to grow them all on many differen media and under varying conditions and, again, a type collection i essential.

Requirements of different laboratories, as regards size and typ of collection, will vary greatly, but there are a number of genera methods and precautions whose consideration is applicable to a cases. The main essentials may be briefly summarized thus :

1. All cultures must be kept alive as long as required.
2. Every culture must be maintained in a state of purity.
3. As far as possible, each species or strain must be kept true t type, that is with all the characteristics it had when firs introduced to the collection.

The chief essential in keeping moulds alive is transfer at suffic ently frequent intervals. Different species, even of the same genu vary much in longevity, the spores of some common moulds remair ing viable for years whilst others lose their power of germinatio after a few months. Some cultures can withstand desiccation an may readily be sub-cultured even after they have dried up to horny mass, whilst others, chiefly those which do not spore freel must be kept on a moist medium and die off if allowed to becom even approximately dry. The frequency with which sub-culturin of a collection is necessary depends very largely, therefore, on th peculiarities of the particular fungi, but it also depends to som extent on external conditions, particularly on the temperature of th place of storage. If kept in a room where the average temperature is 18–20°C., most of the common moulds may be kept alive by transferring two or three times a year, whilst yeasts and related fungi should be sub-cultured about every two months. If the place of storage can be maintained at a temperature of about 10°C., cultures will remain in good condition for a longer time and the majority of species may safely be left eight or nine months between transfers. With such a place of cold storage available it is best to grow sub-cultures in the 25° incubator until characteristic features have developed sufficiently to decide whether the transfers have been completely successful and then to remove the cultures to the 10° storage chamber, where growth continues more slowly. A few species, notably those belonging to the *Aspergillus glaucus* group, grow well at 10°C., some of them better than at 20° Even in these cases the lower temperature is suitable for storage since its chief advantage is that drying out of the medium is retarded. When special accommodation at a low temperature cannot be provided experience will soon show how often the collection should be sub-cultured, but it is always better to err on the side of frequency, since the loss of a valuable culture is very annoying.

As regards choice of media for stock cultures it is best to aim at encouraging growth which is characteristic but not too luxuriant. For maintaining vigour in slow-growing and delicate species wort agar is ideal, but many of the more strongly growing species tend, on this medium, to produce masses of aerial mycelium at the expense of typical fruiting structures. Czapek agar is not so rich a medium as wort and is ideal for a great many species. Potato agar and prune agar are intermediate and are particularly valuable for many of the Mucorales, which completely choke up the culture tubes when grown on wort but grow very poorly on Czapek agar. Most of the dematiaceous fungi keep in good condition on corn meal agar, usually producing abundant spores and comparatively little aerial mycelium. Carrot extract agar is also excellent for a wide variety of fungi. Gelatine media are, on the whole, unsuitable for stock cultures, as the gelatine is readily liquefied by very many moulds. In any collection it is advisable to keep at least two, and preferably three stock cultures of each mould as an insurance against loss by accident or contamination, and these may well be on different media, since a periodical change of diet seems to preserve vigour and character in many species.

It sometimes occurs, on account of delay in sub-culturing or even for no apparent reason, that transfers made in the usual w fail to grow. It is unwise to assume at once that the old cult is dead. A method of saving the species, even when only a f viable spores remain and when the chances are that repeated nee transfers will effect nothing, is to flood the surface of the old cult with a small quantity of sterile liquid medium and incubate. T new medium provides sufficient moisture and nutriment to bri about the germination of any spores which are still alive, after whi sub-cultures may be made in the usual way. Another method to scrape off as much as possible of the old growth in about 3 c of sterile water, then flood this suspension over the surface of suitable agar medium in a Petri dish.

The problem of keeping stock cultures in a state of purity beset with more difficulties than is the task of merely keeping the alive. In the first place it is obvious that sub-culturing a numb of species, one after the other, demands the utmost care and faultle technique. Even, however, when every care is taken, infectio will sometimes occur if the work is done in a highly contaminate atmosphere. Sometimes it is unavoidable that cultures are handle in such a way that clouds of spores are discharged into the ai If this happens in a room where transfers of stock cultures ha to be made, it is well, before starting to sow, to minimize the ris of infection by sterilizing the air, using one of the methods describe in Chapter X.

Even the best workers are troubled by occasional contaminatio of cultures and it is therefore very necessary to be able to recogniz such when it does occur. Infection of a culture by a species of different genius, say the invasion of a *Mucor* by a *Penicillium*, easily seen on casual inspection, but infection by a closely relate species of similar appearance may go unnoticed for a long time an may even result in complete loss of the original and its replacemen by the contaminant. The main essential, the importance of whic cannot be over-emphasized, is that the worker in charge of th collection should keep full records of all species, noting gross appearance at various ages, rate of growth and microscopical appearanc both of the living cultures and of mounted specimens. It is tru that an enthusiastic mycologist in time gets to know all his mould as well as the keen gardener knows all the plants in his garden bu even then, records are more valuable than memory alone and shoul never be omitted or scamped. Periodical examination of the colle tion should be made and, if there is the slightest suspicion of anythin wrong, the particular species should be plated out, fresh cultures being made from typical colonies.

When the culture collection includes species of several different genera it will be found that chance infection due to imperfect methods of transfer is more readily spotted if the species are stored and sub-cultured in random order. For example, number 1 in the collection may be an *Aspergillus*, 2 a *Mucor*, 3 a *Cladosporium*, 4 a *Penicillium*, and so on throughout, with never more than two or three of the same genus bearing consecutive numbers. A collection of species of a single genus, particularly one in which there is little colour variation, is far more likely to carry unnoticed contaminants than is a miscellaneous collection.

Mites are a most annoying pest to have in the culture laboratory and are one of the most serious causes of contamination. A collection of fungi may remain unattacked for years and then, suddenly, scores or hundreds of cultures are found to be infected, but, unless the mycologist is constantly on the look out, and unless he knows what to look for, the depredations may go unnoticed for a long time and may result in wholesale contamination and loss of many species. Fig. 142 is a photomicrograph of a typical mite in a tube culture. The different species vary somewhat in shape and size, but, when once seen, can never be mistaken for anything else. An adult mite is usually about one-hundredth of an inch in length and is thus almost at the limit of unaided vision. When alive and in motion they are, as a matter of fact, readily seen, as small whitish specks, by anyone with normal sight but, if any cultures are known to be infected, or there is any reason to suspect the presence of mites, *all* cultures should be carefully examined under the microscope, using a 2″ objective. The presence of eggs should be particularly noted, as these are more difficult to kill than are the adult mites. Like the adults and larvæ they are pale coloured, but appear to be dark brown when infested cultures are examined by transmitted light. They are surprisingly large, in comparison with the adult mites, and so are readily seen even when lying amongst masses of hyphæ and spores (Fig. 143). The evil the mites do is twofold. In the first place they eat the cultures and, if left unchecked, may even destroy them entirely. In addition, they crawl from one culture to another with spores adhering to their hairy bodies, spreading infection wherever they go. Petri dishes are readily entered and become contaminated with amazing rapidity if once the mites appear in their vicinity. Cultures in tubes are just as readily invaded, since the

mites find no difficulty in crawling through the tightest cotton-w
plugs and according to Thom, they have even been known to
their way through paraffined plugs. If the plugs are a good fit
mites shed most of the adhering spores *en route* to the cultures,
major portion of the damage being then due to their gastrono
activities, but mixing of cultures does occur and is presumably
to spores which pass unchanged through the digestive tract.

The control of mites is not easy. They are brought in with
kinds of raw materials, they may be introduced with new laborat
fittings and apparatus or they may occur in cultures sent fr
other workers, and hence hygienic measures sufficiently stringent
prevent their access to the culture room are difficult to realize
practice. Constant vigilance is necessary, with the prompt appli
tion of suppressive measures at the first indication of an atta
The usual method of killing mites is to expose the infested cultu
to the vapour of some substance which is highly toxic to the mi
and relatively non-toxic to moulds. Jewson and Tattersfield (19
examined a number of possible fumigants and selected pyridine
the most suitable, mites being killed in three to four hours and eg
usually in about sixteen hours. Page and Shafik (1936) tried o
a variety of substances, chiefly with a view to ridding insects
parasitic mites, but they also give the results of a number of expe
ments with cultures of common moulds. They recommend carb
tetrachloride and methyl salicylate and give the concentrations
these and several other fumigants which will kill all stages of mit
without affecting the viability of any of the moulds tested. Pea
(1937) considers *p*-dichlorbenzene to be the safest fumigant, Pet
dish cultures to be exposed to a saturated atmosphere for two hou
and plugged tubes for four hours, the fumigation to be repeated eve
forty-eight hours for twelve days. Crowell (1941) also recommen
"dichloricide" (a trade name for *p*-dichlorbenzene) and states th
it is so innocuous to moulds that spores will readily germinate an
give rise to normal colonies when crystals of the fumigant are place
inside the culture tube at the time of inoculation.

If a readily volatile fumigant is used it is essential that the do
be controlled. As regards pyridine, there seems to be a fairly narro
range of concentrations within which it is effective. If the concen
tration is too low some of the mites survive, and they and the
immediate progeny seem to be completely immune to any concen
tration of pyridine vapour. If a very high concentration is use
from the beginning, or the time of exposure is prolonged, the mite

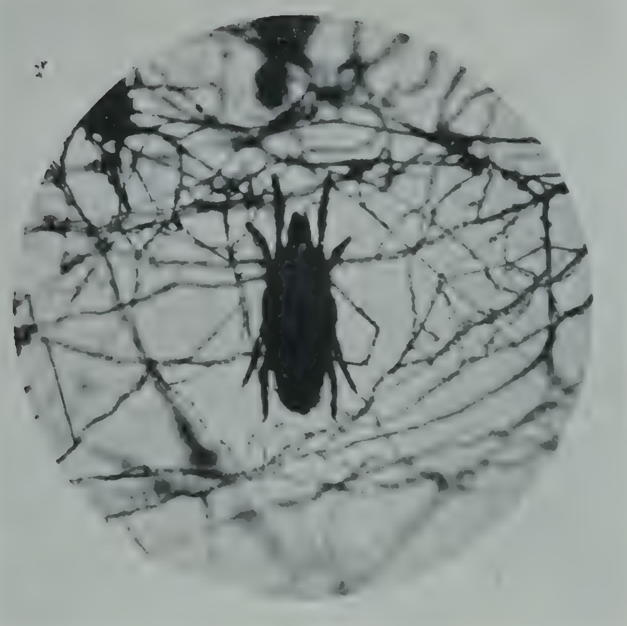

FIG. 142.—Mite (adult) in culture tube. × 100.
The larvæ are somewhat similar in appearance but have only six legs.

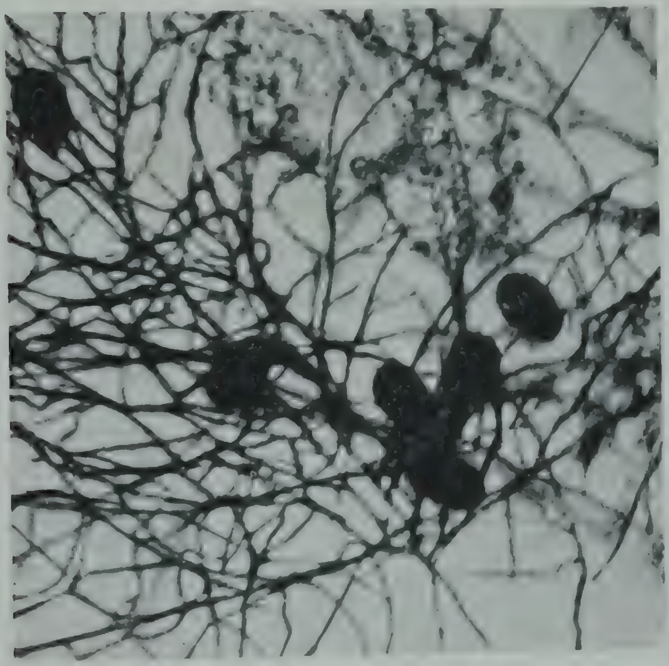

FIG. 143.—Eggs of mite as seen in culture tube. × 100.

are eliminated but many of the cultures are killed also, proba
owing to the fact that pyridine is readily soluble in water and
absorbed by the culture medium. Carbon tetrachloride is insolu
in water and appears to be safer than pyridine, but even this su
stance is toxic to many moulds at high concentrations. The actu
concentrations recommended by Page and Shafik, for an exposu
of at least four hours at 25°C., are: carbon tetrachloride, 0·25 c
per litre of air space in the fumigation chamber; pyridine, 0·05 c
per litre. It is safe to fumigate for twenty-four hours and it is
wise precaution to repeat the fumigation after the lapse of four
seven days, in case any mites or eggs have escaped destructio
Compared with carbon tetrachloride, *p*-dichlorbenzene is onl
slightly volatile and can safely be used in excess to give a saturate
atmosphere.

Whichever substance is used, fumigation should be carried ou
in a vessel which can be tightly closed, as otherwise the letha
concentration cannot be maintained with certainty. Also, none o
the substances is devoid of physiological action and should not b
allowed to escape into the air of the laboratory in any considerabl
amount. If only a few cultures are to be treated they may be stoo
upright in a large bottle, into the bottom of which the necessar
quantity of fumigant is placed. Larger numbers of tubes may b
packed in wire baskets and placed in any convenient receptacle
preferably made of metal. The large-size biscuit tins are admirabl
for the purpose and will easily accommodate at least 100 tubes
Whilst the cultures are being treated the cupboards in which the
are normally stored should also be fumigated, in order to kill an
mites which have lodged in the joints of the woodwork. The easies
way of doing this is to place in each cupboard a few open dishe
containing strong ammonia. The vapour is very penetrating an
highly toxic to all stages of mites, but, since it is also toxic t
moulds, the cupboards must be well aerated before returning th
cultures.

Prevention of infestation is anything but a simple problem
particularly when, as is usually the case, cultures have to be store
in the same laboratory in which all culture work, including examina
tion of infected materials, is carried on. In some laboratories it i
the custom to paint bands of paraffin and rubber tap-grease roun
the rims of the culture tubes, but such tubes are messy to handl
and the treatment is of doubtful efficacy as a method of prevention
for mites have actually been observed to crawl through such

grease band, very slowly, it is true, but with a sure sense of direction. Hansen and Snyder (1939) describe a method of mechanical exclusion which is claimed to give complete protection. Immediately after sowing a culture the cotton-wool plug is discarded and the tube is sealed by a cap of cigarette paper, stuck to the rim by a smear of gelatine containing copper sulphate as antiseptic. It is stated, however, that only one brand of paper (American) was found which excluded mites and at the same time allowed free access of air to the cultures. Barnes (1933) recommends that cultures should rest on small tripods of lead, these standing in dishes of water. The method, which was designed for dealing with comparatively small numbers of cultures in Petri dishes, may be extended to the protection of a culture collection by raising store cupboards on legs which stand in dishes of water or paraffin. By this means it is probable that direct access of mites is prevented, but infestation may still occur in at least two ways. Mites are often transferred on the hands of the worker from infested cultures or from mouldy materials to stock cultures, and it should be the general rule to scrub the hands thoroughly and to swab the work bench with dilute alkali or acid after handling infested or suspect material. Pease (1937) recommends that benches should be swabbed with 50 per cent acetic acid and that fresh culture medium and cultures from stock should never be placed directly on the bench but on special shelves or on flamed tripods. The second possible mode of introduction of mites is by flies. Mites usually have four stages in their life history: egg, larva, nymph (i.e. a sexually immature adult) and adult, but in some of the Tyroglyphidæ, a family which includes the cheese mites and culture mites, the nymph often undergoes a peculiar transformation into what is termed a "hypopus." This has no proper mouth parts and does not feed, it has very short legs and moves but slowly, but it has a number of sucker plates by which it can attach itself to insects for free transport. It is obvious that, in any place in which flies can settle, mites may be deposited by their temporary hosts. Whether infestation by such means does occur will depend largely on whether there is a local focus of infection, but it is certain that, where such a source of mites exists, the maintenance of a culture collection in good condition demands constant and close watch for signs of infestation. Storage of cultures at a low temperature (8–10° C.), if this can be arranged, is a help, since mites are not attracted to cold areas and, in any case, their development proceeds only slowly at low temperatures.

One precaution should always be taken after killing off the mite in infested cultures. Fresh sub-cultures should be made withou delay, and examined carefully for the presence of contaminants. I is advisable to fumigate these sub-cultures as soon as they have mad satisfactory growth and been checked, and to transfer once mor after a short interval.

Finally it remains to be considered how far it is possible to keep moulds, through a series of sub-cultures, true to type. Some moulds vary little or not at all, successive transfers coming up in every way as far as can be seen, identical with the original. Unfortunately on the other hand, a few fungi seem to be decidedly unstable in artificial culture and defy all efforts to maintain their distinguishing features. Between these two extremes are a large number of species which can be kept in good condition only if special precautions are taken. The usual type of deterioration is the increased production, in successive sub-cultures, of sterile mycelium at the expense of fruiting structures. For example, species of *Chætomium* (and other less common Sphæriaceæ) tend to lose the power of producing perithecia; many Dematiaceæ, in particular species of *Alternaria*, *Stemphylium* and allied genera, often produce masses of floccose, whitish or greyish mycelium over a thin basal felt of typical dark-coloured hyphæ, and spores are formed very tardily and sparingly or not at all; some species of *Fusarium* deteriorate very rapidly, growing as masses of ropy, slimy mycelium, with complete lack of typical macroconidia; strains of the *Penicillium luteum* group frequently become entirely mycelial, lacking both perithecia and conidia; even some of the *Aspergilli*, normally amongst the least troublesome of all fungi in this respect, occasionally deteriorate and become almost sterile. To some extent deterioration of this type can be controlled by suitable choice of culture media. Some fungi will grow normally only on substrates containing low concentrations of nutrients. For example, Coons (1916), in an investigation of cultural variations in *Plenodomus fuscomaculans*, found that pycnidia were produced only when the source of carbon was present in less than a definite limiting concentration, and it is not unusual to find that species of *Phoma* show a similar tendency, producing pycnidia only on media containing very little soluble carbohydrate. In contrast to these, some members of the *Aspergillus glaucus* series deteriorate on substrates of low concentration, but are rapidly restored to vigour if grown on culture media containing 20–40 per cent of cane sugar. Some fungi will not grow on synthetic media, requiring certain accessory factors which are found in many vegetable decoctions but which are absent from media compounded of pure salts and sugars.

Another cause of deterioration of certain fungi is lack of light. Some species appear to grow equally well in light or dark, but in many others sporulation is stimulated by strong light, and it is always advisable to try the effect of short exposures to bright sunlight on any cultures which are showing undue development of sterile aerial mycelium. A number of species of *Penicillium*, particularly the Biverticillata, responded well to this treatment, even old degenerate strains of *P. luteum* producing typical conidiophores after stimulation by a few hours' exposure. Many of the pathogenic *Helminthosporia* do not spore at all in artificial culture under ordinary conditions, but Weston (1933) has shown that *H. avenæ* can be induced to form normal conidia if cultures in quartz Petri dishes are submitted to one or two short irradiations from a quartz mercury vapour lamp.

A cause of variation inherent in the fungus itself has been brought to light by Hansen (1932, 1938) and termed the "Dual phenomenon." The spores of many fungi are bi- or multi-nucleate, the nuclei being of two types, one promoting mycelial growth (M), the other production of spores (C). Cultures originating from conidia containing nuclei of the C type only remain typical through a series of sub-cultures and produce abundant fruiting structures. Spores containing only M nuclei give rise to mycelial cultures, with scanty spore production, whilst cultures from single spores containing both M and C nuclei (MC) are of intermediate type. Mixed cultures from several spores, including CC, MM and MC types, produce, by anastomosis, mycelium containing both kinds of nuclei. The method of obtaining homotypic sporing cultures is to make a fairly large number of single-spore cultures. When these reach maturity they will probably show marked differences in degree of freedom of sporulation and amount of sterile mycelium produced. Those which produce spores most freely and show least tendency to development of floccose mycelium are selected and from them further series of single-spore cultures are made. This process of selection is continued until a culture is obtained which produces spores of one kind only, the CC type, all of which will develop into similar, freely sporing colonies. Hansen and Snyder (1944) state that the well-known instability of the strain of *Penicillium notatum* used for production of penicillin can be explained as an example of the dual phenomenon, but Baker (1944) maintains that the conidia of this species a predominantly uninucleate.

Another type of deterioration is, fortunately, not common, bu when it does occur, is more serious in that it usually results in tot loss of the species. Growth in successive transfers remains fairl typical but becomes less and less vigorous until the vitality of th fungus seems to dwindle entirely away. If the preservation of suc a strain is desirable it is necessary to carry out a fairly extensiv cultural investigation, trying the effect of different sources of carbo and nitrogen, of the addition of minute amounts of metals other tha the usual combination (zinc, for example, seems to be essential in a few cases and acts as a stimulant in others), of adjusting the medium to different reactions, of growing the fungus at various temperatures and so on, until a set of conditions can be formulated which will ensure vigorous growth.

Mutation is not uncommon in most genera of fungi and provides a fascinating subject of study. It is commonly evidenced by what is known as "sectoring," a more or less circular colony of the mould showing one or more fairly clean-cut sectors, which vary from the rest of the colony in such characters as colour, texture and degree of sporulation. Sectoring may often be induced by abnormal conditions, such as a temperature near the maximum for growth, or the presence of small amounts of toxic substances. The new type of growth, in these cases, may either be stable or may revert back to the original on restoring to normal conditions. Some species regularly give rise to true mutants when grown in the normal way and, if the new strain happens to be of more vigorous habit than the parent, the latter may be entirely swamped unless the change is noticed in good time and the two forms separated.

The phenomenon of heterothallism, as a case of apparent degeneration of cultures, has already been discussed in Chapter I.

A final form of variation from type, which the biochemist knows only too well, concerns, not the appearance or structure of the fungus, but alteration in chemical activity or loss of the power to produce some particular metabolic product. Two examples, from amongst the many recorded in the literature, may be cited to illustrate this type of change. Wehmer, in 1918, described a new species of *Aspergillus* which gave good yields of fumaric acid when grown on glucose media and which was therefore named *A. fumaricus*. In a later paper, 1928, he reported that his fungus no longer produced fumaric acid but gave gluconic acid instead. Birkinshaw and

Raistrick (1931) describe the production, by four strains of *Penicillium* related to *P. spinulosum* Thom, of a substituted toluquinone which gave to the culture medium an intense purple colour. All the strains, when freshly isolated, gave appreciable amounts of the specific product, but all gave gradually decreasing yields, and after repeated sub-culturing, refused to produce the least trace of the substance. This kind of variation gives rise to serious difficulties when an attempt is made to utilize the metabolic activities of fungi for the large-scale production of substances of industrial importance. Before any such process can become a commercial proposition it is necessary to carry out much preliminary work in order to ensure that any tendency to loss of activity can be completely controlled.

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CHAPTER XIII

THE CONTROL OF MOULD GROWTH

The growth of moulds in undesirable places is the cause of almost incalculable loss in industry and of less serious, but still extensive, damage in many households. It is therefore desirable that the available means for combating their ravages should be as efficient as possible and generally known.

Much may be done to minimize trouble from moulds in the factory by strict attention to hygiene. Damp and dirty walls, corners of floors and ceilings which are not easily cleaned, or waste organic material left lying about are all liable to develop patches of mould and act as reservoirs of infection, the spores being carried to all parts of the factory by air currents. Leaky steam-pipes or water condensed from process steam on ceilings and parts of machinery may cause drips on to valuable material, creating conditions eminently suitable for the growth of fungi. Suitable precautions to prevent infection from such sources as these are obvious. In some cases, where the neighbourhood is excessively dusty or where there is a known local source of airborne infection, it may be advisable to purify the air entering the building.

Even with ideal factory conditions, however, it is seldom possible to prevent entirely the access of mould spores to factory products. Latent infection may be carried in raw materials, complete sterility of the air in a work-room is almost impossible to achieve owing to air currents created by movements of materials and workpeople and the latter may introduce spores in large numbers on their clothing. Therefore, even if damage by moulds within the factory be completely prevented, there remains the problem of ensuring that products remain in good condition after they leave the factory and until they eventually reach the consumer. There are three main lines of attack on this problem:

1. To ensure continuous sterility by preventing the access of mould spores to an already sterile or sterilized product.
2. To arrange that an industrial product is kept, or keeps itself, in such physical condition that growth of moulds is limited or prevented entirely.

3. To limit mould growth by means of toxic substances, usua
known as antiseptics.

Sterilization Methods. The first method is of very limit application but is usually, and should be always, effective in ca where it can be carried out. The best-known application of t method is, of course, the food-canning industry. In the canning meat, fish and certain vegetables, the main object of sterilizati is to destroy bacteria but, since moulds are less resistant to he than are the majority of bacteria, any cooking operation which w destroy the latter will also automatically kill any moulds presen In the fruit-canning industry, however, high temperatures and lor cooking have to be avoided, as they detract from the appearan of the products. Fortunately the acidity of most fruits is of grea assistance in suppressing bacterial growth and processing can ther fore be conducted at temperatures below 100°C. As already state in Chapter IV, one fungus, *Byssochlamys fulva*, has caused co siderable trouble in the fruit canneries owing to its ability to witl stand a temperature only a little below the maximum attained i processing, necessitating greater care being taken to ensure tha all parts of the contents of the cans reach the safe temperature (se Olliver and Rendle, 1934).

One branch of the food industry, the packing of fruit for expor applies the principle of isolation in a special way. The mould causing rot of fruits are specialized parasites and thus, if the acces of spores of *Penicillium digitatum* and *P. italicum* to citrus fruits ca be prevented, the main cause of spoilage is removed. A good dea of work has been done on the sterilization of citrus fruits by mean of antiseptic washes, chiefly solutions of borax or boric acid. A fai measure of control is thus obtained, but a small proportion of fruit still remain infected. If packed without further precautions infectio may spread and result in serious loss, but, if the fruits are wrappe in waxed or chemically treated papers, any infected fruits may b extensively rotted and covered with a thick growth of mould withou infecting any other fruits in their vicinity. A good account of th control of citrus moulds is given by Fawcett (1936). Wrapping o apples has similarly diminished to a remarkable extent losses du to *Penicillium expansum* rot. It is not unusual to find, in a case o wrapped apples, one or two fruits which are nothing but a mass o brown rottenness, whilst the rest are quite unaffected.

A method of partial sterilization which has received considerabl attention during recent years utilizes the lethal properties of ultra-violet radiation. It has been found that both mould spores and bacteria are rapidly killed by exposure to a source of light rich in ultra-violet rays, although it is doubtful how far the effect is due to the direct action of the rays and how far to the action of the ionized oxygen produced in the neighbourhood of the source of light. James (1936), for example, claims that direct irradiation is unnecessary and that air circulation during the treatment is beneficial, indicating that it is ionized oxygen which is the potent factor. In any case the effect is exerted only on the surface of the irradiated material, since neither ultra-violet light nor ionized oxygen can penetrate far into most kinds of matter. This is of little consequence for such materials as bakery products, which are usually sterile as they leave the oven and become infected only on the surface during cooling. Such goods, after irradiation and warping in irradiated paper, are virtually sterile throughout and remain in good condition until they reach the consumer. The chief applications of the method at present are to various foodstuffs and tobacco, its use for many other materials which undoubtedly could be sterilized in this way being limited by questions of cost and difficulties of sterile packing.

Physico-Chemical Methods. In attempting to limit mould growth by physico-chemical means the most important factor which must be controlled is the amount of available moisture. Spores of fungi cannot germinate without moisture and, therefore, if the amount of water in any material can be kept below a certain critical point, fungoid growth will be entirely prevented. This critical point varies considerably for different materials and must be determined experimentally in each individual case. The reason for such variation is that the absolute amount of water in any material seldom corresponds with what may be termed the available amount, the latter depending on the chemical consitution and physical state of the particular substance. For example, the "safe" limit of moisture in wool is much higher than the amount allowable in cotton, the difference being accounted for by their very different attractions for water. If dry samples of pure wool and pure cotton are exposed to the same atmosphere the wool will take up approximately twice as much moisture as the cotton and, leaving out of account differences due to chemical composition, the two samples will be approximately equally liable to mildew, or, if the respective percentages of moisture are below the respective safe limits, will be equally resistant. According to Galloway (1935) the minimum relative humidity permitting growth varies from 75 per cent to 95 per cent for di ent species of moulds. Adequate protection, therefore, is obtai only if materials are stored in an atmosphere of R.H. less t 75 per cent.

The method of obtaining the necessary data for control of m growth by this method is, first, to determine the relationship betw atmospheric relative humidity and moisture content of the partic material over any desired range of temperature, then to ex samples infected with mould spores to atmospheres of predetermi humidities. In this way is found the lowest relative humidity, hence the lowest percentage of moisture in the material, at wh growth of moulds commences. The infection may be that natur occurring on any particular raw material, or, preferably, the may be made with single organisms which are known to cause dam in practice. A simple method of conducting such tests in the lab tory is to suspend small samples over solutions of calcium chlor of known strength, contained in stoppered bottles or desiccat A series of such containers can be accommodated in an incuba in order to obtain accurate control of temperature. The follow table will be found useful by those wishing to undertake tests this kind.

Grams $CaCl_2$ in 100 g. H_2O.	Density g./ml. at 15° C.	Relative Humidity, per cent.				
		0° C.	10° C.	20° C.	25° C.	30° C
15	1·115	92·8	91·0	89·7	89·5	(90·0)
20	1·150	87·7	87·2	87·5	86·0	(85·6)
30	1·213	76·8	77·8	77·4	76·2	(76·3)
40	1·272	66·7	67·4	66·3	65·9	66·1
50	1·325	56·9	55·6	54·6	55·3	56·3
60	1·374	43·8	44·8	43·9	45·2	46·7
65	1·396	,,	40·0	,,	,,	,,
70	,,	,,	,,	34·1	35·7	38·0
80	,,	,,	,,	,,	29·9	30·9

Figures underlined indicate the presence of solid salt.
Figures in brackets are extrapolations.

Laboratory tests give useful and necessary information but, attempting to apply the results in practice, complications may ari chiefly due to temperature variations. If any hygroscopic substan is freely exposed to the air the amount of moisture in the substan when equilibrium has been reached, is a determinable function of the relative humidity of the air, but a great many industrial products are transported, stored and marketed in packages which are airtight or through which diffusion of air is extremely slow. The great majority of solid substances tend to lose moisture as the temperature rises, whereas the capacity of air for holding moisture is enhanced with increasing temperature. Two questions then arise, for the answering of which we have far from adequate data at the present time. How does the moisture distribution inside an airtight package, which contains solid material together with a comparatively small weight of air, vary as the temperature is changed? Also, can spores of moulds acquire the moisture necessary for germination from a humid atmosphere, even though the substrate with which the air is in contact is comparatively dry? An interesting paper by Galloway (1935) attempts to answer the second of these questions. He made use of the fact that cellulosic materials, exposed to an atmosphere of definite humidity, contain different percentages of moisture according as they are being dried from a wet state or moistened from the dry state. He was thus able to use substrata which contained different percentages of water when exposed to the same atmosphere, but were otherwise identical, and also materials, otherwise identical, which contained the same percentage of moisture when exposed to atmospheres of different humidities. The experiments showed that atmospheric moisture is more effective than moisture in the substrata for bringing about the germination of mould spores. This fact may possibly supply the explanation of a number of mysterious cases of damage by moulds, when the spoiled material has been apparently too dry to permit growth, but it is impossible to generalize until we have an answer to the first of the above questions. There is a promising field for research along these lines.

Another factor which tends to lower the permissible limit of moisture in stored products is the possibility of local variations of temperature within the package. If one side of an airtight container be exposed to a source of heat, such as direct sunlight, a ship's boilers, or heating pipes in a warehouse, whilst the opposite side is kept comparatively cool, it is reasonable to expect that migration of moisture will occur, and the extra water acquired by the cool portions of the package may be sufficient to cause the germination of dormant spores. Against the possibility of damage arising in this way a manufacturer has no certain means of protection. The

utmost he can do is to allow as large a safety margin as possib
the total moisture content of his product and to urge merchant
take suitable precautions during transport and storage of the s
A practice which is finding increasing use for goods shipped to
tropics is to pack the materials in airtight containers along wi
suitable receptacle containing a desiccant. The best dehydra
agent is undoubtedly silica gel, as it is clean in use and can rea
be reactivated by heat.

Another method of preventing mould growth, one, however, wh
can be used only in special cases, is to limit the amount of *availa*
moisture in moist materials by arranging that there is a high conc
tration of soluble matter, thus ensuring that the water pres
is in the form of a solution of very high osmotic pressure. T
best-known example of this method is the manufacture of fr
preserves. It has been found from experience that the concentrati
of sugar in the finished article must be close to 65 per cent in ord
to ensure sterility. With amounts of sugar only slightly less th
this a few cosmopolitan species of moulds, notably members of t
Aspergillus glaucus series and *A. candidus*, can grow quite we
Pickling in brine is another process relying for its efficacy on t
same phenomenon.

A method of protecting certain foodstuffs from bacterial attac
namely, cold storage, is effective in preventing damage by mould
only if the temperature is considerably below freezing-point.
fairly large number of species of moulds can grow at 0°C., or eve
at slightly lower temperatures, and a few will grow at −6°C.

Antiseptics. The control of mould growth by the use of antisept
substances has been practised, or at least attempted, for some con
siderable time. It is only in recent years, however, that organize
research on the subject has put the practice on a scientific basi
explained many failures of the past, and made available new sub
stances of high antiseptic value and general applicability.

It should be stated at the outset that antiseptics are not necessaril
fungicides. Fungus spores are often very resistant to toxic substance
and most substances which will kill them are unavailable for use i
industrial products because they are corrosive, give off objectionabl
vapours or are highly poisonous to human beings. Antiseptic
merely inhibit growth and their action is limited by a number
factors, such as the availability of nutriment present along with th
antiseptic, amount of available moisture, concentration of antisepti
substance and, in some cases, time. In certain cases, such as woo

preservation, it is possible to use powerful antiseptics in high concentration, and effect complete protection, but often there are technical objections to the use of mould preventatives in really efficacious amounts, as well as questions of cost, and the most that can be achieved is to prevent mould growth under more or less normal conditions of use and storage. The concentration of antiseptic required to prevent growth usually increases rapidly as the humidity of the atmosphere increases, and there are a number of antiseptics which are effective at normal humidities but which become entirely useless when the relative humidity exceeds 95 per cent. There are many antiseptics which, at very low concentrations, actually stimulate the growth of fungi. With somewhat higher concentrations germination of spores is delayed and, with still larger amounts, completely inhibited. A study by Morris (1927) of a large number of antiseptics showed some interesting differences in behaviour when these were incorporated in substrata otherwise favourable to mould growth. There were naturally large differences in the amounts of the various antiseptics required for a definite retardation of growth but, apart from this, it was found that the curves relating concentration of antiseptic to degree of inhibition were of different types. With some substances the effect was strictly, or almost strictly, proportional to concentration, whilst with others there seemed to be a maximum effect, short of complete inhibition, which could not be exceeded by the use of higher concentration of the antiseptic.

An important point which is often overlooked is what may be termed the specificity of antiseptics. Different species of fungi react differently to any particular antiseptic. A substance may give adequate protection so long as the potential infection is limited to certain species and may fail completely if spores of another species are introduced. For example, salicylic acid is a very efficient preventive of the growth of most of the Dematiaceæ and of many *Penicillia*, but can actually be utilized as a source of carbon by *Aspergillus niger*. Zinc chloride is a popular and normally efficient antiseptic, but, in concentrations generally used, has no effect on *Aspergillus terreus*. Morris found that salts of thallium are of high efficiency with most of the species used in his tests but very much less effective with *Cladosporium herbarum* and *Alternaria tenuis*. Hexamine, on the other hand, has comparatively low antiseptic value with most fungi but is particularly effective against the few moulds which can grow in presence of thallium. It is advisable, therefore, when considering the question of an antiseptic for protecting any particular product, to find first of all what are the usu species of moulds which infect the product, and then test o various antiseptics with these species.

The actual testing of antiseptics, so as to give results of valu is by no means a simple matter. A usual method of obtaining preliminary valuation is to grow a number of species of moul in the laboratory, on media containing varying percentages of th antiseptic and to measure growth rates. The basal medium use may be one of the usual agar culture media or, better, a mediu containing the particular material to be protected as sole source nutriment, the material being used either in the form of a stiff past or as a fine suspension in a plain agar gel. To begin with, a numbe of media are prepared containing say 0·01, 0·05, 0·1, 0·5, and 1· per cent of the antiseptic respectively. A series of Petri dishes ar prepared with equal quantities of the various media, at least tw and preferably more dishes for each combination of species an concentration of antiseptic, and single colonies of the various specie are planted centrally in the dishes. Rates of growth are recorde as colony diameters, usually with comments on the type of growth, such as density of mycelium and degree of sporing. Fuller details of the method are given by Morris (1927). In this way a fair comparison between different antiseptics may be achieved and some idea obtained of the amounts required to suppress growth. The method, although often the only one available for preliminary tests, is, however, open to the objection that such test media contain a very high percentage of water and the conditions are thus not comparable with those obtaining in practice. It is therefore necessary, before the true value of an antiseptic can be assessed, to make further tests under works conditions. In some industries, such as the manufacture of toilet preparations, incorporation of antiseptic with product can be carried out with very small batches of material and a number of tests can be run at comparatively little expense. Even here, however, the preliminary tests, as outlined above, are advisable, as they give results quickly, whereas experiments with industrial products for export may take months. With such materials as textiles, where the antiseptic is added to size or dressing, a single factory experiment may be costly and dislocating to normal work, so that it is absolutely necessary to obtain as much information as possible from laboratory tests.

It remains to give some details of the substances in common use as antiseptics in industry.

INORGANIC SUBSTANCES

Zinc chloride was originally suggested for the preservation of wood by Sir W. Burnett in 1838 (E.P. 7747). For this purpose it has been entirely supplanted by creosote preparations and copper compounds, but it has found extensive use in the cotton industry as an ingredient of the size added to the warp threads to assist weaving. It has to be used in fairly high concentrations, usually reckoned as 10 per cent on the weight of adhesive substances used in the size, but it is reasonably cheap and has the additional advantage of adding weight to the yarn. It is only moderately effective and, as stated above, may fail entirely when the goods are infected with certain *Aspergilli*, notably *A. terreus*.

Chromium compounds. Chromic oxide, Cr_2O_3, is an excellent antiseptic when used in fairly high concentration. The so-called mineral khaki, extensively used for military fabrics, is composed of oxides of chromium and iron, and materials dyed with this mixture show a very high resistance to attack by moulds. Chromium oxide can be used alone in cases where a pale blue-green colour is not objectionable, the amount required corresponding to approximately 1 per cent of chromium. For materials which have to come in contact with the skin it is essential that the impregnation be carried out in such a way that not more than a trace of chromate ion is left in the fabric, as hexavalent chromium is liable to cause dermatitis. Chromium fluoride is used for proofing woollen goods. About 1 per cent on the weight of the material confers not only protection against moulds but also a high degree of resistance to attack by moths and other insects.

Copper compounds. Copper sulphate has been suggested for use in various trades, but has never been popular, possibly because of a much-repeated statement in trade literature that moulds have been found growing on strong solutions of the salt used for electrotyping. On the whole it is more efficient than zinc chloride but is unreliable in the same way as the latter and is more expensive. Copper naphthenate has found extensive use, either alone or mixed with other antiseptics, for the preservation of timber. This and other organic compounds of copper, such as the oleate and ricinoleate, are used for the protection of fabrics which come into intimate contact with the soil, such as sandbags. These compounds are very effective antiseptics against soil-borne bacteria and certain soil fungi.

but are much less effective against common moulds. If used materials which are exposed to the weather they are, like inorganic compounds of copper, gradually leached out and. addition, they accelerate the destructive effect of strong sunli on cellulose. Copper carbonate in various forms, such as Burgu and Bordeaux mixtures, is widely used for spraying plants as preventive of mildew. Its use was originated by Pasteur at a ti when the vineyards of France were threatened with ruin by t rapid spread of the vine mildew.

Mercury salts are little used, although quite effective, account of their well-known poisonous nature and the fact th the metal is readily deposited from solutions of the salts by oth common metals.

Thallium salts are powerful antiseptics but are little use partly owing to high cost and partly due to the publicity giv to one or two cases of accidental poisoning some years ag Actually, although the price of thallium carbonate averages abo 60 shillings per pound, the cost in practice compares very favou ably with that of other antiseptics because of the low concentratio required.

Sodium silicofluoride behaves somewhat erratically but h enjoyed considerable popularity and constantly appears on th market under a variety of fancy names. One disadvantage fo many purposes is its low solubility in water. It has been found t be one of the best antiseptics for the protection of raw rubber, th material being soaked in a 0·25 per cent solution.

Boric acid and Borax. Both substances are of low toxicit to most moulds but find a limited application in some industrie where highly poisonous substances are debarred. They are use either singly or in combination, as a wash for citrus fruits to minimiz attack by the rot organisms, *Penicillium digitatum* and *P. italicum* A number of papers relating to their use will be found in the variou agricultural journals published by the exporting countries.

ORGANIC SUBSTANCES

The number of compounds having antiseptic value is very large Many, however, are too expensive for most purposes, whilst other have very limited applications owing to pronounced colour, odour volatility or instability, with the result that there are only a comparatively small number of substances of real value to industry.

Creosote finds its main application for the preservation of wood. It gives considerable protection when merely brushed on, but, for adequate effect in timber which has to withstand severe conditions, it is applied under pressure, sometimes following vacuum, or at a temperature sufficiently high to boil the sap out of the wood. Its use for railway sleepers, pit props, piles and telegraph poles has meant increased safety for large numbers of workers as well as an enormous saving in the cost of replacements. Its somewhat objectionable odour, even when partially purified as in certain proprietary preparations, its dark colour and action on the skin, prevent its more general use.

Organic Mercurials. A large number of organic compounds of mercury are known and all are very potent antiseptics. The alkyl compounds are appreciably volatile at ordinary temperatures and are mainly used in agriculture for the control of seed-borne parasitic fungi. They are applied as dusts containing large amounts of inert fillers, in order to ensure even distribution over the seeds and are mostly sold under trade names. The aryl compounds are not volatile except at high temperatures and a number of them have been suggested for protection of industrial materials. Colin-Russ (1940) tested nineteen antiseptics for their value in protecting leather and shoe-lining materials, the best being "merfenil" (phenyl mercuric nitrate). He states that this is effective at a concentration of 0·00175 per cent, whereas the amount of shirlan required for equal protection is 0·1 per cent. Marsh and Duske (1942) recommend phenyl mercuric acetate as the ideal antiseptic for military fabrics, the procedure being to soak the material in a 1 : 2,000 solution, wring out and dry at room temperature. It is stated that the antiseptic is cheap to apply, that it is not readily washed out and that there is no danger of dermatological action. It is unusual, however, to carry out large-scale drying of processed fabrics at room temperature and Marsh and Duske give no data regarding the effect on the antiseptic of drying by normal methods. A serious disadvantage of the organic mercury compounds is that, although they are not ionized, they are readily decomposed, at temperatures above about 40° C., by contact with common metals such as copper, aluminium and iron. This precludes their use in goods which are dried, after processing, by passing over heated metal cylinders. As regards the question of dermatological action, there is at present insufficient information available to decide whether there is any danger attending the use of these substances in fabrics which come into intimate contact with the skin. The alkyl compounds are known to have a vesicant actio and must be handled carefully, but the aryl compounds are muc less active in this respect and the concentrations necessary fo suppression of mould growth are extremely low.

Phenol is probably more efficient for preventing bacterial rathe than mould growth. Nevertheless, it shows a fairly high toxicit to most fungi. In an acid medium its odour is distinctly noticeabl and objectionable for some purposes, whilst as alkaline phenate its antiseptic value is lower.

Chlorphenols. The halogen-substituted phenols are very much more efficient antiseptics than phenol itself. Pentachlorphenol is used in a variety of products, such as leather, cordage, packing papers, and even in some cosmetics. Tri- and tetrachlorphenols are equally effective but are not at present available in quantity in this country. All these compounds are appreciably volatile at ordinary temperatures and have somewhat pronounced odours.

Paranitrophenol is a valuable antiseptic for use in products in which its colour is no detriment. The free acid has a pale, though definite, yellow colour, whilst the alkaline salts are intensely yellow. It is used at a concentration of approximately 0·1 per cent, calculated on the weight of substance to be protected. It is largely used for treatment of rubber, the material being soaked for several hours in a 0·15 to 0·3 per cent solution. It is also an excellent antiseptic for leather, one of the most liable of all materials to attack by moulds.

Salicylic acid has been much recommended but has never proved entirely reliable in use. When tested in the laboratory against a representative collection of moulds, it has been found to inhibit some species completely, even in low concentration, whilst a few species, notably the black *Aspergilli*, are little or not at all affected, and, as stated above, can actually utilize the acid in a culture medium containing no other source of carbon.

Shirlan, the anilide of salicylic acid, has been patented as an antiseptic by the British Cotton Industry Research Association (1928). It was selected by them, after testing a very large number of organic compounds, as the most efficient antiseptic for use in the cotton industry, which demands an antiseptic which is colourless, odourless, non-volatile in steam and unaffected by other materials used in sizing and finishing or by contact with common metals. It is now finding increasing use in other industries because of its high efficiency and reasonable cost. It is marketed in two forms, the free acid (Shirlan), which is only slightly soluble in water, and the readily soluble sodium salt (Shirlan NA). It has been found that the minimum amount for adequate protection is approximately 0·04 per cent of the weight of substrate. This quantity is sufficient in what may be termed normal circumstances, but it is advisable to use double the amount if goods are likely to be exposed to a damp atmosphere.

Other Organic compounds. The number of compounds which show antiseptic action to a greater or less degree is very large, but, apart from those already mentioned, few have been used except for special purposes. Diphenyl, for example, is effective against only a few species of moulds and finds its chief use for impregnating the paper used for wrapping citrus fruits. Colin-Russ (1940) recommends β-naphthol as an antiseptic for leather, but more recent work has shown that it does not give protection to goods exposed to very high humidities. A number of terpenes and related compounds are good antiseptics but, for most purposes, are too volatile and too expensive. A recent introduction to the list of useful substances is 2 : 3-dichloro-1 : 4-naphthoquinone (Ter Horst and Felix, 1943). It is stated to be effective not only as an antiseptic for fabrics but also as a protection for plants against soil-borne fungal infection.

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CHAPTER XIV

INDUSTRIAL USES OF FUNGI

As an offset to the incalculable damage caused by fungi there are a number of industrial processes in which the biochemical activities of moulds are turned to good account. Only a brief discussion of these will be entered into here and the reader is referred to the literature cited for fuller accounts. A few such processes have already been indicated in previous chapters but are mentioned again for convenience of reference.

Alcoholic Fermentation and Mould Enzymes. There are two industries, brewing and baking, which use processes of great antiquity, both dependent on the fact that yeasts convert sugar into alcohol and carbon dioxide. In the brewing of alcoholic beverages alcohol is the important product, whilst the carbon dioxide, once allowed to escape as useless, is now a valuable by-product, being collected, solidified and marketed as "dry ice." In baking, on the other hand, the production of alcohol is incidental and it is the carbon dioxide which is valuable, causing the dough to rise and giving lightness to the bread. Both industries have an extensive literature of their own and need no further description here.

Yeasts secrete the enzyme zymase, which effects the conversion of sugar to alcohol, but lack diastase, the enzyme which breaks down starch to sugar. There are, however, a number of fungi which secrete a whole range of enzymes and can ferment complex carbohydrates without a preliminary saccharification. In certain processes for the production of alcoholic beverages from starchy materials moulds alone are used, but in others moulds effect the saccharification of the starch, after which a yeast is allowed to act on the sugars produced, since, although the mould can complete the conversion to alcohol, the yield is better when yeast is used for the second stage. Fitz in 1873 was the first to show that alcohol is produced by a mould, *Mucor mucedo* (later identified as *M. racemosus*), and other workers have since shown that similar results are obtained with several other species of the same genus. In the "Amylo" process, which is still used in many countries in more or less modified forms, *Mucor Rouxii* was first used but was later replaced successively by various species of *Rhizopus*. Good general accounts of earlier practice are given by Lafar (1903) and Wehmer (1907), whilst more mod methods are described by Galle (1923). Various Mucoraceæ are a active agents in the so-called starters, used for initiating alcoho fermentations and marketed in various Eastern countries, Chin rice being the best-known example.

Japanese “Koji” differs from other starters in making use of qu a different group of fungi, strains of the *Aspergillus flavus-ory* series. In addition to the production of Saké, the national alcoho beverage of Japan, a number of other industries utilize the enzym secreted by *A. flavus-oryzæ*. Soy sauce is made by fermentation soya beans, the starter being produced by growing the fungus cooked beans, usually mixed with some other starchy material to a rapid growth. As a result of an extensive study of the enzym of this group of moulds Takamine has introduced into commerce number of products of high enzymic activity, sold under a variet of names such as “Takadiastase” and “Polyzime,” and used f the dextrinization of starch and the desizing of textiles. Takamin himself (1914) has summarized his work in a short paper, whic should be consulted for further details.

Mould-ripened Cheese. The manufacture of mould-ripene cheese is another industry of unknown origin. Until recently suc cheeses were associated with particular districts, such as the cave of Roquefort and the town of Stilton, and the methods used wer entirely empirical, the distinct flavours of certain brands bein dependent on a combination of slight local peculiarities in the qualit of the curd and of a natural local infection of a particular strai of the all-important mould. It was not until 1906 that Thom' studies of the cheese moulds led to a proper understanding of th ripening process and, even at the present day, when countles cheeses are being made by pure culture methods, there are certai local blue-veined cheeses whose production is a happy acciden rather than a matter to be determined at choice, the nature an source of the marbling being a complete mystery to those who mak the cheeses.

There are two distinct types of mould-ripened cheese, the sof cheeses of the Camembert and Brie types and the green or blu veined cheese, of which Roquefort, Gorgonzola and Stilton are th best known. In the first type the moulds concerned are *Penicilliun camemberti* Thom and *P. caseicolum* Bainier, the two giving somewhat different flavours. The curd is made into cakes 3 to 4 cm thick, salted on the surface and either inoculated with spores o the fungus or placed in an infected room. The initial moisture in the cakes is 55 to 60 per cent and the air in the ripening room is maintained at a temperature of 50–60° F. and a relative humidity of about 88 per cent. Freedom from infection by undesirable moulds depends on maintaining both temperature and humidity within fairly narrow limits. The mould grows on the surface of the cheese and gradually softens the whole mass of curd, the process requiring about four weeks for completion. The most serious source of infection and spoilage is *Scopulariopsis brevicaulis*, which gives to the cheese an ammoniacal taste and odour.

For production of the marbled cheese the raw curd is pressed so as to leave irregular cracks and channels. In the pure culture method a sterile curd is inoculated, before pressing, from a culture of the mould (usually on bread), and is later aerated from time to time during the ripening process by piercing with wires. In the older process natural infection is relied on, and the success of the method depends on the fact that few moulds other than *P. roqueforti* can grow with the small amount of oxygen contained in the narrow air spaces in the curd, and hence the chance of contamination with an undesirable species is small. Although some workers consider that distinct species are concerned in the ripening of the various types of cheese, Thom has shown that, for all practical purposes, all the strains may be regarded as one species. We still know little, however, of the effect of using different strains under otherwise identical conditions.

Oxalic and Citric Acids. There are a few modern processes which have been developed as the direct result of purely academic investigations into the biochemical activities of moulds. The first worker to make substantial progress in this field was C. Wehmer, who, in 1891, showed that oxalic acid is a definite fermentation product of *Aspergillus niger* and made an extended study of its production from various sugars. Oxalic acid, however, has never been made commercially by this method because the more usual chemical methods are cheaper. In 1893 Wehmer described the production of citric acid by two species of moulds which were made the types of a new genus, *Citromyces* (now included in *Penicillium*). Other investigators have found that citric acid is a fermentation product of many species of *Penicillium*, but in no case is the yield sufficiently good to enable this method of production to compete with the established process of extracting the acid from citrus fruits. In 1917, however, Currie showed that, in the fermentation of sugar by black species of *Aspergillus* (the *A. niger* group) there is a dis lag between the acidity of the medium due to oxalic acid and t acidity, the difference representing citric acid. Methods were wo out for suppressing the formation of oxalic acid and increasing yield of citric acid, the chief essentials being a high initial con tration of sugar (about 15 per cent), low concentrations of ammon nitrate as source of nitrogen, and an acid reaction of the cult medium, *p*H about 3·5. However, a number of difficulties have to be overcome before such a process could be worked commercia Sterilization of culture media is an expensive operation; an abund supply of air is required and must be supplied in a sterile conditi or alternatively, the fermentation must be carried out in shal layers of liquid with free aeration and suitable protection fr infection, requiring expensive plant and expert supervision; a perhaps most important of all, any organism is liable to be erra in its behaviour, making the question of yields uncertain. A la number of patents have been taken out in this field, but the act methods at present in use have not been made public in th entirety. Citric acid is certainly being made successfully in Engla Belgium, the United States of America and Japan, in quantit sufficient to make these countries almost or quite independent imported acid made from citrus fruits.

Gluconic acid is formed from sugars by the action of a lar number of species of moulds, chiefly species of *Aspergillus* a *Penicillium*, and a considerable amount of work has been carri out in the United States of America in an attempt to develop large-scale production by mould fermentation (May *et al.*, 192 Herrick and May, 1928; May *et al.*, 1929). Whether the proce is yet being worked successfully on an industrial scale is, howev doubtful. The acid is used chiefly as the calcium salt, in place calcium lactate, in medicinal and food preparations.

Gallic acid was obtained by Calmette in 1902 by fermenting clear extract of tannin by means of an organism which he nam *Aspergillus gallomyces*, the fungus being grown submerged in a we aerated and agitated liquid. Modifications of Calmette's proce have been used, and probably are still being used, both in Euro and in America, but exact data are not available.

Glycerol. About the middle of last century Pasteur (185 showed that glycerol is formed in small amounts during the alcoho fermentation of sugar by yeast, the maximum yield being abo 3 per cent of the sugar consumed. During the First World War th acute shortage of fats in Germany led to an investigation of the process with a view to increasing the production of glycerol. It was found that the yield may be much improved by carrying out the fermentation in an alkaline medium and still more so by adding to the culture medium sodium sulphite, which has the additional advantage that it inhibits the growth of bacteria without affecting the activity of the yeast. The yield of glycerol obtained was about 25 per cent of the sugar used and actually something over 1,000 tons per month was produced by this method. After the War details of the process were published by Connstein and Lüdecke (1919). Later the technical production of fermentation glycerol was developed in the United States of America, using a special strain of yeast and adding sodium carbonate to the fermenting liquor up to a final concentration of approximately 5 per cent (Connstein and Lüdecke, 1924).

Fats are synthesized from carbohydrates by a large number of fungi. Until recently, however, only one species, a yeast usually known as *Endomyces vernalis* Ludwig, was of more than theoretical interest, it having been used for the large-scale production of fat in Germany towards the end of the 1914–18 War. According to Haehn and Kinttof (1924), the essentials for successful working are a well-aerated medium rich in sugar and poor in nitrogen. They claim yields up to 25 per cent of the dry weight of the yeast cells and 30 per cent of the sugar consumed, also that the yield can be increased by the introduction of alcohol vapour in the air supply.

A number of American workers (Lockwood *et al.*, 1934; Ward *et al.*, 1935) have investigated the production of fat from glucose by a large number of species of moulds. The largest amount is given by *Penicillium javanicum* van Beyma, which gives yields of fat up to more than 40 per cent of the weight of the mycelium, the best results being obtained with a medium containing 40 per cent of glucose. The process of production and isolation of the fat has been carried out with semi-large-scale apparatus and could no doubt be worked industrially in any emergency which created a shortage of fats from the usual sources.

An account of a very thorough investigation of the possibilities of large-scale production of fat is given by Fink, Haehn and Hoerburger (1937). They describe in detail experiments carried out at the Institut für Gärungsgewerbe, both with *Endomyces vernalis* and with a number of other organisms. They find that the best yield of fat (30 per cent of the sugar) is given, under favourable conditi by *E. vernalis*, and that *P. javanicum* is unsuitable owing to low yields calculated on the starting material. Promising resu were obtained with *Oidium lactis*, which, though giving a lo overall yield than *E. vernalis*, gives a better yield on a time proc tion basis. *O. lactis* has the further advantage that it is practica proof against infection and so can be grown on unsterilized mater However, the general conclusion of Fink *et al.*, is that none of processes so far described is suitable for peace-time production fat, owing to the high running costs of the plant required.

Proteins are synthesized by a number of fungi, usually as c constituents, more rarely in the substrate. In particular, the yea *Saccharomyces cerevisiæ* and *Torulopsis utilis* contain very conside able percentages of protein of high nutritive value. Since yeasts c be grown on the large scale with ammonia as the sole source nitrogen and with cheap carbohydrate, such as molasses, as sour of carbon, it is not surprising that, during the War, a good deal interest has been taken, both in this country and in Germany, yeasts as a possible supplement to the normal diet. Neither count has had a surplus of suitable by-product carbohydrate but in Ge many the manufacture of yeast for food was considered of suc importance that thousands of tons were made, using sugar obtaine by acid hydrolysis of wood.

In England a large amount of experimental work has been carrie out, but manufacture has not been undertaken on account of th difficulty of importing the necessary carbohydrate. On the basis the English experiments, however, manufacture has been starte in Jamaica, where molasses and surplus raw sugar are available large amounts.

The organism selected for the large-scale experiments was *Tor lopsis utilis*, as this is easier to grow and gives a more palatab product than *Saccharomyces cerevisiæ*. The yeast was subjected t chemical stimulation, in the hope of obtaining mutants with mor desirable characteristics than the parent strain and, as a result, tw interesting new varieties were produced. *T. utilis* var. *thermophil* grows well at 39° C., a temperature considerably above that suitab for growth of the parent strain, and can be used for manufactur in hot countries (Thaysen, 1943). *T. utilis* var. *major*, described b Thaysen and Morris (1943), has cells approximately twice the siz of those of the parent.

The manufactured product, known as "Food Yeast," is straw-coloured powder, containing about 45 per cent of protein, as well as valuable amounts of the B group of vitamins. It is palatable, with a slightly nutty taste and often imparts an improved flavour when added to other foods, such as soups, stews, pastry, biscuits and bread. Full details of the Jamaica experiment are given in a booklet published by Colonial Food Yeast, Ltd. (1944).

A useful review of the possible methods of production of protein by micro-organisms is given by Fawns (1943).

Vitamins. One of the best sources of the vitamin B complex is yeast, which is, in fact, one of the very few readily accessible foods containing all the four or more factors which comprise the vitamin B group. The increasing recognition of the importance of these vitamins has led food manufacturers to put on the market a number of preparations of high potency, made from yeast extracts or autolysed yeast. The best known of these, used as a source of vitamin B in numerous nutritional experiments, is Marmite.

Ergosterol, the precursor of vitamin D, is synthesized by a number of moulds as well as by yeasts (Pruess *et al.*, 1931, 1932 *a* and *b*; Birkinshaw *et al.*, 1931) and there are on the market at the present time a number of preparations of irradiated ergosterol, mostly made from yeast.

Although ascorbic acid (vitamin C) has not yet been obtained as a metabolic product of fungi, substances closely related in chemical structure are produced by certain *Penicillia* (Clutterbuck *et al.*, 1935; Birkinshaw and Raistrick, 1936) and it is not outside the bounds of possibility that the vitamin itself will be found in due course.

Antibacterial Substances. In 1929 Fleming found, as a contaminant of a bacterial culture plate, a species of *Penicillium* which showed the remarkable property of completely inhibiting the growth of certain bacteria. The original colony of the mould, later identified as *P. notatum* Westling, was surrounded by a zone which was clear of gram-positive cocci but in which *B. influenzæ* was growing normally. It was shown that filtrates from cultures of the mould on broth showed the same selective inhibitory effect and it was suggested that culture filtrates could be used for the routine isolation of *B. influenzæ*, a slow-growing organism which is otherwise difficult to isolate when accompanied by more robust species, and might be of value for the treatment of wounds. Clutterbuck *et al.* (1932), showed that Penicillin is produced in good yield when the mould is grown on a purely synthetic medium, a slight modification of the well-known Czapek-Dox solution. It was found that the active principle could be extracted from the metabolism soluti by ether and that it is very unstable at pH 2 or below.

More recently Florey and his collaborators at Oxford (Chain *et* 1940; Abraham *et al.*, 1941) have shown that Penicillin is bacter static, but not bactericidal, *in vivo* as well as *in vitro*, that it is inactivated by pus, that it has no action on leucocytes at conce trations which are effective against bacteria and that it is non-tox They prepared solid concentrates which were of high potency, thou still containing only a small percentage of Penicillin, and carri out a number of successful clinical trials. The results obtained the Oxford workers showed clearly the value of Penicillin as therapeutic agent of unique properties, and led to the initiation a very extensive programme of research, not only at Oxford but many other establishments in this country and abroad. Pu Penicillin has now been obtained and has been found to be reasonab stable in the form of its salts (the sodium and calcium salts a most commonly used), but its chemical constitution has not y been determined and there seems to be no immediate hope of i production by synthesis. In the meantime much work has bee done on the best methods of production by fermentation. A pape by Coghill (1944) describes a number of lines along which researc has proceeded in America. Yields have been enormously increase by careful selection from single-spore cultures of the mould and b improvements in culture media. At one time it was supposed tha Penicillin is produced by only one strain of *P. notatum*, that isolate by Fleming in 1929, but it has now been found that a number strains belonging to the *P. chrysogenum-notatum* group can synthesize the drug and that Penicillin, or something very similar, i produced also by a strain of *Aspergillus flavus*. One strain of *notatum* has been found which gives good yields when grown i submerged culture, thus permitting the use of deep fermentatio vessels containing large volumes of medium instead of the numerou small vessels required for surface culture. Another procedure whic has been developed in America is to grow the mould on moist sterilized bran.

Until recently the whole production of Penicillin was devoted t research, clinical and chemical, and to the treatment of battl casualties, but, owing to improvements in production methods, limited amount is now available for civilian needs. The very extensive literature relating to Penicillin, published up to the end of 194 is summarized in a British Medical Bulletin (1944).

The world-wide interest in Penicillin has stimulated the search for other mould products with antibacterial properties, in particular for substances capable of inhibiting species of bacteria which are resistant to Penicillin. Very large numbers of species of fungi have been examined in various laboratories but, whilst many show some degree of antibacterial activity, the number which produce potentially useful substances is very small and it is probable that the chief value of many of the results obtained will be in indicating the types of chemical structure which are associated with antibacterial activity. Preliminary findings have been published by White (1940) on *Aspergillus flavus*, by Glister (1941) on an unnamed species of *Aspergillus*, and by Wiesner (1942) on *A. clavatus*, whilst a fuller account is given by Chain, Florey and Jennings (1943) of a substance named Helvolin, obtained from *A. fumigatus mut. helvola* Yuill. A number of strains of *A. fumigatus* have been shown to produce antibacterial substances. Waksman *et al.* (1942) described Fumigacin, and Oxford and Raistrick (1942) showed that the substituted toluquinones Fumigatin and Spinulosin (the latter being obtained also from *Penicillium spinulosum*) are highly active but are unstable in solution. Several species of *Penicillium*, apart from *P. notatum* and *P. chrysogenum*, show fairly high activity. Raistrick and Smith (1941) found that Citrinin, formed in good yield by *P. citrium* Thom, is active, though to a much less degree than Penicillin, against gram-positive organisms. Oxford Raistrick and Smith (1942*a*) have shown that Penicillic acid, most readily obtained from the metabolism solutions of a strain of *P. cyclopium* Westling grown on Raulin-Thom solution, inhibits the growth of many gram-negative organisms as well as the gram-positive cocci. A full account of the antibacterial properties of these two substances, *in vitro*, has been given by Oxford (1942). Patulin, obtained from *P. patulum* and *P. expansum* (Raistrick *et al.*, 1943), and identical with Claviformin obtained by Chain, Florey and Jennings (1942) from *P. claviforme*, was reported by Raistrick *et al.* (1943) to be a cure for the common cold, but later large-scale trials have not substantiated the original findings. (Stansfield *et al.*, 1944.) Puberulic acid and puberulonic acid, two compounds whose constitutions, in spite of much work, have not yet been finally determined, have been obtained from many species of *Penicillium* and their activities against a wide range of bacteria described by Oxford, Raistrick and Smith (1942*b*).

Miscellaneous Products of Moulds. During recent years intensive investigations on the biochemistry of moulds have been carried out in several countries, with results which are of gr theoretical interest and which may become the basis of importa industrial processes in the future. In particular, H. Raistrick a co-workers (1931–44) have shown that a number of common moul can produce from glucose a bewildering variety of complex orga compounds, many of which have not yet been synthesized chemists. The majority of these are of purely academic interest the present time, but the metabolic products of one genus of fun appear to have a wider importance. Some species of *Helminth* *sporium* synthesize from glucose substituted anthraquinones of type not readily made by purely chemical means (Charles *et al* 1933; Raistrick *et al.*, 1933, 1934), the yield in the case of *H* *gramineum* Rabenhorst being as much as 30 per cent of the tota weight of mycelium. The main factor which is likely to imped the large-scale production of these valuable dyestuffs intermediate is the extreme slowness of the fermentation, a difficulty which wi probably be overcome by intensive cultural work.

Useful summaries of present knowledge of the biochemistry o moulds are given by Birkinshaw (1937) and Raistrick (1940). Othe general literature relating to industrial fermentations is cited i Chapter XV. Ramsbottom (1936) deals with the uses of fungi i a much wider sense.

Fungi and the Soil. Amongst the uses of fungi, though hardly to be classed as an industrial use, should be mentioned the rôle they play in maintaining the fertility of the soil. Along with bacteria they are responsible for breaking down all kinds of dead organic matter to form humus, returning to the soil and the atmosphere the materials removed by other living things. Without such scavengers other forms of life would soon become extinct.

Fungi occurring in the soil are, however, of importance in other connections. A number of plant parasites live in the soil and attack economically important plants through the roots or at ground level. Of these, species of *Pythium* are responsible for enormous losses as the cause of damping-off disease of seedlings. As mentioned on p. 210, *Trichoderma lignorum* (syn. *T. viride*) and *Gliocladium fimbriatum* both produce a substance, gliotoxin, which inhibits the growth of *Pythia* and gives a considerable degree of protection to young plants. Van Luijk (1938) investigated a number of species of moulds, which normally inhabit the soil, as to their inhibitory effect on various species of *Pythium*. He found that the metabolism solution obtained by growing a strain of *Penicillium expansum* on a synthetic medium containing sugar was highly active in protecting seedlings from attack by *Pythia*, although inoculation of the soil with the *Penicillium* itself had no effect. The active substance was not isolated by van Luijk, but Anslow, Raistrick and Smith (1943) have shown that it was most probably patulin, isolated by them from a number of strains of *P. expansum* and, in better yield, from *P. patulum*.

Another most interesting activity of soil fungi has been described by Drechsler (1937), who found that a number of soil-inhabiting Hyphomycetes can trap and destroy eel-worms. Some of the species concerned are difficult to grow on ordinary culture media, but thrive in presence of soil bacteria and eel-worms. On the mycelium are formed numerous loops, which act as nooses, tightening as soon as an eel-worm tries to pass through, and later sending out branches to draw nourishment from the captive. More recently Drechsler has published several other papers describing a large number of fungi, belonging to several genera, which act in much the same manner.

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OXFORD, A. E., RAISTRICK, H., and SMITH, G. (1942*a*). Anti-bacterial substances from moulds. Part II. Penicillic acid, a metabolic product of *Penicillium puberulum* Bainier and *Penicillium cyclopium* Westling. *Chem. nnd Ind.*, **61**, 22–4.

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CHAPTER XV

MYCOLOGICAL LITERATURE

The following list of publications is not intended to be in any sense a complete bibliography. Apart from a number of works used for identification of fungi, the literature cited includes only such as is considered to be useful to a student whose interest extends to the more general aspects of mycology and who wishes to keep abreast of modern knowledge. Anyone who desires an exhaustive list of publications relating to any particular branch of the subject will find full bibliographies in the various monographs mentioned below.

Unfortunately a number of the most important mycological publications are to be found in only a few English libraries. Some of these may be obtained on loan through the various county libraries, but a few works of reference cannot be borrowed. These may, however, be consulted, by special permission, at the libraries of the British Museum (Natural History), the Imperial Mycological Institute, Kew, and the various universities (if available there).

1. General Works used for Identification of Fungi

Clements, F. E., and Shear, C. L. (1931). Genera of Fungi. New York: The H. W. Wilson Co.

This is a second edition, much enlarged, of a book published by Clements in 1909 and intended primarily as a key to Saccardo's Sylloge. Keys are given to the known genera, with references to Saccardo, but there are no separate generic diagnoses, and, in consequence, the book is not so easy to use as Lindau's (see below). Although written in English a fair knowledge of Latin is essential to anyone using the book as the keys are liberally besprinkled with Anglicized Latin terms. There is, however, a fairly extensive glossary of Latin and English terms. The classification is, in several respects, unorthodox and must be followed with caution by the beginner. A number of plates of illustrations are included, but these cover only a small selection of genera.

Corda, A. C. I. (1837–54). Icones fungorum hucusque cognitorum. Prague.

A somewhat rare book containing beautiful illustrations of a large number of fungi, not always accurate in the light of present-day knowledge owing to the comparative crudity of microscopes in Corda's time, but often helpful.

ENGLER, A., and PRANTL, K. (1897–1900). Die natürlichen Pflanze familien. I Teil, Abteilungen I and I*. Leipzig.

The work is accessible in most botanical libraries and is much used taxonomists. It gives descriptions and illustrations of most gener but no separate descriptions of species.

LINDAU, G. (1922). Kryptogamenflora für Anfänger. Band II. I mikroskopischen Pilze. 2nd ed., Berlin: Julius Springer.

Published in two parts—Part 1 including Myxomycetes, Phycom cetes and Ascomycetes; Part 2 including Rusts, Smuts and Fun Imperfecti. This is a most useful and comparatively inexpensi book, not so widely known and used as it deserves to be. It giv keys to all the families and genera, with separate generic diagnos and descriptions of several species in most cases. The lists of specie are, necessarily, in a book of this size, incomplete and do not alwa include the most common forms, but for tracking down a fungus t its genus the book is extremely good. The illustrations are not s good. They are all from line drawings, small, often very sketchy an not infrequently inadequate. No legends are given and magnifica tions are not stated, so that often a fair knowledge of structure i required in order to know what they are supposed to represent. They are, nevertheless, at times very useful.

RABENHORST, L. (1884–1921). Kryptogamenflora von Deutschland, Oesterreich und der Schweiz. Leipzig: Eduard Kummer.

A series of many volumes, illustrated, including not only the Fungi but other Cryptogams. Some portions are not yet completed. Band I, Die Pilze, is in ten volumes, of which the most useful to a student of moulds are Vol. 4, Phycomycetes, by A. Fischer, 1892, and Vols. 8 and 9, Hyphomycetes, by G. Lindau, 1904–10.

"Rabenhorst" is to be found in many libraries which do not contain a set of Saccardo's Sylloge and is a most useful work of reference. The lists of species are fairly complete up to the dates of publication and include most of those commonly occurring.

SACCARDO, P. A. (1882–1931). Sylloge fungorum omnium hucusque cognitorum. 25 volumes, to be continued. Pavia, Italy.

This unique work includes descriptions, in Latin, of all known species of fungi. It was originally planned in eight volumes, which were completed in 1889. By this time, however, the literature of descriptive mycology was growing to such an extent that a series of supplementary volumes was issued at intervals of a few years. Since Saccardo's death in 1920 the publication has been continued by A. Trotter.

"Saccardo" is to the mycologist very much what "Beilstein" is to the organic chemist, often indispensable but always, of necessity, several years out of date. Unfortunately the work is very expensive and complete sets of volumes are to be found in only a few libraries in this country.

From the point of view of handy reference it is unfortunate, but unavoidable, that the species of a single genus are scattered through several volumes. The work is, however, well indexed and, with the

requisite knowledge of Latin, not difficult to use. The main weakness is that many of the descriptions are, through no fault of Saccardo's, incomplete or merely fragmentary. Even at the time when Saccardo began the compilation it was impossible for one man to have first-hand knowledge of all the thousands of species of fungi. To a large extent, therefore, the treatment is non-critical, many of the diagnoses being merely transcriptions into Latin of original descriptions which were totally inadequate.

Saccardo, P. A. (1877–86). Fungi Italici autographice delineati. Pavia, Italy.

A collection of illustrations, mostly coloured, of a very large number of fungi, including most of the common moulds.

2. Monographs and Important Papers dealing with Particular Genera of Moulds

Alternaria

Elliott, J. A. (1917). Taxonomic characters of the genera Alternaria and Macrosporium. *Amer. Jour. of Bot.*, **4**, 439–76.

The best account so far of the genus *Alternaria*. Species of this genus are not difficult to identify if the author's conceptions of species limitations are adopted.

Aspergillus

Thom, C., and Church, M. B. (1920). The Aspergilli. Baltimore: The Williams and Wilkins Co.

Thom, C., and Raper, K. B. (1945). Manual of the Aspergilli. Baltimore: The Williams and Wilkins Co.

The Thom and Church monograph has for long been the standard work on the genus but is now out of print. The new book by Thom and Raper brings the taxonomy up to date and incorporates a number of recent detailed studies of certain groups. It includes a large number of illustrations, some in colour.

Chætomium

Chivers, A. H. (1915). A monograph of the genera *Chætomium* and *Ascotricha*. *Mem. Torrey Bot. Club*, **14**, 155–240.

This is the best treatment of *Chætomium* up to date. Full descriptions of all species are given, with numerous illustrations.

Fusarium

Wollenweber, H. W., and Reinking, O. A. (1935). Die Fusarien. Berlin: Paul Parey.

Wollenweber's monograph of the genus, the first part of which was published in 1931 (*Zeit. f. Parasitenkunde*), has not been finished. In the meantime, the present book gives a somewhat simplified account of the genus and, although it is too soon to judge adequately, it offers hope of mycologists being able to identify Fusaria isolated from sources other than diseased plants.

Wollenweber, H. W., Sherbakoff, C. D., Reinking, O. A., Johann and Bailey, A. A. (1925). Fundamentals for taxonomic stu of Fusarium. *Jour. Agric. Res.*, **30**, 833–43.

This paper still remains of importance to anyone working w Fusaria. It gives explanations of terms and criteria, and detail culture media, a good deal of the information not being include Die Fusarien.

Mucorales

Zycha, H. (1935). Kryptogamenflora der Mark Brandenburg. Band V Pilze II, Mucorineæ. Leipzig: Gebrüder Borntraeger.

This monograph is mainly taxonomic and includes descriptions of known species of all the genera of Mucorales. The treatment of two commonest genera, *Mucor* and *Rhizopus*, is excellent. The bo is likely to supersede all previous publications used for identificati of moulds belonging to this order.

Penicillium

Thom, C. (1930). The Penicillia. London: Baillière, Tindall & Cox.

This is undoubtedly the most complete and satisfactory account this genus up to date. It is not an easy book to use but is indi pensable to anyone whose work entails regular identifications Penicillia. A fair number of new species of Penicillium have be described since 1930, but the majority of these can be traced throug the catalogues issued by the various national collections of culture

Yeasts

Lodder, J. (1934). Die anaskosporogenen Hefen. Erste Halfte. Ve handelingen der Koninlijke Akademie van Weterschnappen Amsterdam, Afd. Naturrkunde, (Tweede Sectie) Deel **32**, 1–25

Stelling-Dekker, N. M. (1931). Die sporogenen Hefen. *Ibid.*, Deel **2** 1–547.

These two publications form parts of a comprehensive treatise on th Yeasts and yeast-like fungi, by workers at the Centraalbureau voo Schimmelcultures, Baarn, Holland. To those who wish to identif organisms belonging to these very difficult groups they are indispensable.

3. BOOKS ON GENERAL AND SYSTEMATIC MYCOLOGY

de Bary, A. (1887). Comparative Morphology and Biology of the Fung Mycetozoa and Bacteria. English translation. Oxford Univ Press.

Although written fifty years ago, this is still one of the best account of systematic mycology.

Bessey, E. A. (1935). A Text-book of Mycology. Philadelphia: P Blakiston, Son & Co.

This covers much the same ground as other modern books on th subject but contains in addition an excellent bibliography under th title “Guide to the literature for the identification of fungi.”

ITZPATRICK, H. M. (1930). The Lower Fungi—Phycomycetes. New York: The McGraw-Hill Book Co.

A very full account of the comparative morphology of this class of fungi.

ÄUMANN, E. A., and DODGE, C. W. (1928). Comparative Morphology of Fungi. New York: The McGraw-Hill Book Co.

Although regarded as a standard work on the subject, this is not a book for the beginner and should not be attempted by the student until he has acquired a fairly good working knowledge of mycology.

UILLIERMOND, A., and TANNER, F. W. (1920). The Yeasts. New York: John Wiley & Sons, Inc.

An English translation by Tanner of Les Levures, published by Guilliermond in 1912, considerably enlarged and brought up to date in collaboration with the author. It has long been regarded as the standard systematic treatment of the sporing yeasts. (See Stelling-Dekker.)

WYNNE-VAUGHAN, H. C. I., and BARNES, B. (1937). The Fungi. 2nd ed. Cambridge Univ. Press.

For the beginner this is a much more readable book than Gäumann and Dodge. The classification adopted differs somewhat from that of Gäumann and also from that of Bessey.

AMSBOTTOM, J. (1929). Fungi. London: Ernest Benn (sixpenny series).

A popular account of the Fungi from many aspects. It is a most useful introduction to the subject and should be read by everyone commencing the study of mycology.

TELLING-DEKKER, N. M. (1931). *Loc. cit.*

This is the most recent treatment of the sporing yeasts. The classification differs somewhat from that of Guilliermond and Tanner.

4. BOOKS ON APPLIED MYCOLOGY

ALLOWAY, L. D., and BURGESS, R. (1940). Applied Mycology and Bacteriology. 2nd ed. London: Leonard Hill, Ltd.

A good introduction to the study of general microbiology. Gives a brief account of micro-organisms, including methods of study, and includes a number of chapters on the importance of moulds and bacteria in particular industries.

ARSHBERGER, J. W. (1917). Mycology and Plant Pathology. Philadelphia: P. Blakiston, Son & Co.

Primarily a book on plant pathology, this has been much used as an introduction to the study of moulds in default of a work devoted specifically to such. It gives a brief account of systematic mycology and also, in a series of appendices, a number of keys (not always quite accurate) to *Mucor*, *Aspergillus* and *Penicillium*.

ENRICI, A. T. (1930). Molds, Yeasts and Actinomycetes. New York: John Wiley & Sons; London: Chapman & Hall.

The consideration of moulds and yeasts from an industrial standpoint is subordinated to the medical aspect of mycology. As an introduction

to the study of pathogenic fungi and of that difficult class of organi
the Actinomycetes, the book can be recommended.

Lafar, F. (1904–14). Handbuch der technischen Mykologie. Zw
Aufl. Jena: Gustav Fischer.

A series of five volumes dealing with the rôle of micro-organis
industry. Vol. 1 (1904–7) deals with general morphology and phy
logy of organisms used in fermentations; Vol. 2 (1905–8) with
food industries; Vol. 3 (1904–6) with water, soil and manure; V
(1905–7) with special morphology and physiology of yeasts,
mould fungi; Vol. 5 (1905–14) with the mycology of beer, spi
wines, fruits, vinegar, leather and tobacco. Recognized as a stand
work on the subject.

Lafar, F. (1898–1910). Technical Mycology. English translation of
German edition, by C. T. C. Salter. London: C. Griffin & Co

Vol. 1 (1898) deals with bacterial fermentations; Vol. 2, Part 1 (19
Part 2 (1910) with fermentations brought about by fungi. Whilst
so full or up to date as the later German edition, this gives a g
account of the history of the fermentation industries and of
technique of last century.

Prescott, S. C., and Dunn, C. G. (1940). Industrial Microbiology. N
York: The McGraw-Hill Book Co.

A full account of the uses of yeasts, moulds and bacteria in ma
facturing processes, including brief sections on the morphology a
classification of the three groups of organisms. The treatment
mould fermentations, including both those of practical importa
and those of merely theoretical interest, is the fullest yet publishe
collected form. Methods of testing antiseptics and disinfectants, a
the disposal of microbiological wastes are described in appendice

Smith, H. F., and Obold, W. L. (1930). Industrial Microbiology. Lond
Baillière, Tindall & Cox.

Gives accounts, many of them very brief, of industrial proces
utilizing bacteria, yeasts and moulds, including some very mod
methods.

Thaysen, A. C., and Bunker, H. J. (1927). The Microbiology of Cel
lose, Henicelluloses, Pectin and Gums. Oxford University Pre

Thaysen, A. C., and Galloway, L. D. (1930). The Microbiology of Sta
and Sugars. Oxford University Press.

These two books give descriptions of a large number of organis
including bacteria, Actinomycetes and moulds, and summarize
results of researches into the rôle of these organisms in the deco
position of the various complex carbohydrates.

Various Authors (1930). Industrial Fermentations. *Ind. and E*
Chem., **22**, 1148–1225.

This is a series of papers presented at a symposium held at Cincinn
in September 1930, and dealing with both bacterial and mou
fermentations. It is a very valuable résumé of modern practi
(Obtainable as a separate reprint.)

5. Miscellaneous

Ainsworth, G. C., and Bisby, G. R. (1943). A dictionary of the Fungi. Imperial Mycological Institute.

A most useful book, which lists all generic names of fungi published up to the end of 1939, with synonyms, distribution and number of known species for each genus. There are explanations of mycological terms, common names of fungi, short notes on well-known mycologists and many other interesting details concerning important groups of fungi.

Lists of new genera and species described since 1939 are given in a series of supplements to the Review of Applied Mycology (*q.v.*).

Dade, H. A. (1943). Colour Terminology in Biology. *Mycolog. Papers*, No. 6. Imp. Bur. Mycology.

The accurate description of colours of fungi has always been a difficulty to mycologists, particularly in the preparation of diagnoses of new species. Dade's paper lists all the useful terms (and many which should be avoided), in both Latin and English, and gives precise definitions.

6. Journals and Periodicals

The following list does not include all the journals devoted to mycology, but only those which regularly contain papers of interest to the student of moulds and which are reasonably accessible.

Annales Mycologici. Berlin.

Contains original papers, mostly on taxonomy of all classes of fungi, and lists of new literature but not abstracts.

Biological Abstracts. Baltimore, Md., U.S.A.

"Published under the auspices of the Union of American Biological Societies, with the co-operation of biologists generally." This is a continuation of *Botanical Abstracts*, published 1918–26. It is issued monthly, October to May, and bi-monthly June to September, and covers all branches of biological sciences. Abstracts are classified and many of them are written by the authors of the papers abstracted.

Bulletin Trimestriel de la Société Mycologique de France. Paris.

A quarterly journal with a preponderance of papers on taxonomy. Includes abstracts.

Hedwigia. Dresden.

Issued monthly. Contains original papers, relating to Cryptogamic Botany in general and to plant pathology, and excellent abstracts. A half-yearly supplement contains lists of titles of new literature and book reviews.

Journal of Agricultural Research. Washington, D.C. Government Printing Office.

Published twice monthly; two volumes per year. Although it is concerned primarily with all branches of agriculture, a considerable number of papers on taxonomy of moulds have appeared in this journal.

Mycologia. Lancaster, Pa.

This was originally, 1885–1908, *The Journal of Mycology.* I published bi-monthly for the New York Botanical Garden and been since 1933 the official organ of the Mycological Societ America. It covers all branches of mycology with a preponder of papers on taxonomy. No abstracts.

Mykologische Zentralblatt. Jena.

A journal of abstracts, unfortunately issued only from 1912 to 1

Review of Applied Mycology.

Issued by the Imperial Mycological Institute, Kew, and publis monthly. A journal of abstracts only, covering all branches applied mycology but not pure mycology. A series of suppleme published half-yearly, lists all newly described genera and specie

Transactions of the British Mycological Society. London.

Four parts per year are issued, often two parts together. Conta original papers on all aspects of mycology. No abstracts.

Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankhei Zweite Abt. Jena.

Issued monthly, with two volumes per year. Contains original pa in German and English, including a fair number of taxonomy moulds, and classified abstracts in German. (*Erste Abt., Original* concerned with medical and veterinary bacteriology.)

Apart from the journals devoted to mycology, papers on fu are frequently published in the various botanical journals and journals devoted to particular industries, whilst articles on appli mycology are to be found in numerous trade periodicals. All these, however, are adequately covered by the various journ which publish abstracts.

INDEX

Page numbers in italics refer to diagnoses of genera and species, those in heavy type to illustrations. Authors' names are printed in capitals.

ABRAHAM, E. P., 245
Absidia, 20, *31*
A. ramosa, **26**
A. spinosa, **26**
Achlya, **6**
Acrostalagmus, 80
Actinomyces, **62**, 67
Actinomycetes, 66
Aeration of cultures, 203
After-fermentation of beer, 53
Agar culture media, 170
  preparation of, 170
AINSWORTH, G. C., 259
Air in factories, purification of, 225
  necessity of, for moulds, 202
Airtight packages, moisture distribution in, 229
Alcohol, manufacturers of, 27, 31, 44, 239
  utilization of, by yeasts, 47, 56
Alcoholic fermentations, 41, 48, 239
ALCORN, G. D., 194
Algæ, 1, 2
Alternaria, 40, 72, **86**, **87**, *89*
  deterioration of cultures of, 220
  taxonomic literature of, 255
A. brassicæ, **86**
A. tenuis, 4, 5, **87**
  tolerance to thallium of, 231
AMANN, J., 192
Amylo process, 27, 31, 239
Amylomyces (*Mucor*) *Rouxii*, *27*
Antheridium (definition), 8
Antibacterial substances from moulds, 245
Antiseptics, specificity of, 231
  stimulation of growth by, 209
  testing of, 232
  use of, 230
ANSLOW, W. K., 248
Apophysis (def.), 31
Aposphæria violacea, *64*
Apothecium (def.), 33
Apparatus, general, 164
Apples, control of mould on, 226
  rot of, 154
Arsenic, gaseous compounds of, 133
  poisoning due to *Scopulariopsis*, 133
Artefacts in microscopic preparations, 196
Artichokes, fermentation of, 44
Ascocarp (def.), 33
Ascogena (section of *Penicillium*), 158
Ascomycetes, 4, 11, 12, 14, 33
  conidial stages of, 59
Ascorbic acid, 129, 244
Ascotricha, 39
Ascus (def.), 11, 33
Aspergillaceæ, 34
Aspergillosis, 111
Aspergillus, 16, 71
  citric acid production by, 242
  deterioration of cultures of, 220
  determination of species of, 100
  distinction from *Penicillium*, 99
  foot-cells in, 99, **101**
  generic diagnosis of, *98*
  gluconic acid production by, 242
  key to species of, 103
  production of sterigmata in, 99, **101**
  taxonomic literature of, 255
  temperature optima of species of, 208
A. amstelodami, **106**, *107*, *108*
A. atropurpureus, *120*
A. candidus, 100, 103, **118**, *119*
  growth under dry conditions of, 230
A. carbonarius, *120*
A. Chevalieri, 17, **106**, *107*, *108*
A. cinnamomeus, *120*
A. clavatus, 103, *111*, **113**, 246
A. conicus, *111*
A. effusus, *124*, **126**
A. flavipes, 103, **118**, *119*
A. flavus, 100, 247
A. flavus-oryzæ group, 103, *124*, **126**
  industrial use of, 240
A. fumaricus, loss of biochemical activity in, 223
A. fumigatus, 103, **110**, *111*, 247
  temperature limits of, 208
A. fumigatus mut. *helvola*, 247
A. gallomyces, 242

A. giganteus, *111*, **114**
A. glaucus series, 4, 5, 39, 59, 99, **101**, 103, *104*, **105**, **106**
  growth under dry conditions of, 209, 230
  temperature optima of, 208, 213
A. gracilis, *108*
A. herbariorum, 107
A. luchuensis, *120*
A. luteo-niger, *120*
A. nidulans, 60, 101, 103, *111*, **115**
A. niger, *119*, **121**
  citric acid production by, 98, 129, 241
  oxalic acid production by, 98, 241
  Penicillium disease of, 161, **206**, 211
  Raulin's medium for, 174
  utilization of salicylic acid by, 231, 236
A. niger series, 103, *119*
A. ochraceus, 103, *123*, **125**
A. oryzae, industrial uses of, 98
A. penicilloides, *108*
A. penicilloides group, 103, *108*
A. phœnicis, *120*
A. pulverulentus, *120*
A. repens, **101**, **106**, *107*
A. restrictus, **106**, *108*, **109**
A. ruber, **105**, *107*
A. schiemanni, *120*
A. sulphureus, 103, *123*
A. Sydowi, 103, *112*, **116**
A. tamarii, 99, 103, *123*
A. terreus, 103, *112*, **117**
  tolerance to zinc chloride of, 231, 233
  utilization of chlorine by, 202
A. ustus, **101**, 103, *119*
A. versicolor, 103, *112*, **116**
A. Wentii, 16. 103, **122**, *123*
Asperula, (sect. of Penicillium), 144
Asporomyces, 52
Asymmetrica (sect. of Penicillium, 134, 142
Asymmetrica-funiculosa, 152
Asymmetrica-velutina, 142
Atmospheric humidity, control of, 228
  utilization of, by moulds, 229
Authors' names appended to names of species, 17
Autoclaves, 167
Available moisture in substrata, 227, 230

Bacteria, 2
Bacteriostatic action of moulds, 245
Bailey, A. A., 256
Bainier, G., 107
Baker, G. E., 222
Bakers' yeast, 48
Bakery products, control of mo
  on, 227
Baking, 239
Barnard, J. E., 190, 199
Barnes, B., 33, 219, 257
Basidia (def.), 12
Basidiomycetes, 4, 12, 14, 52
Basidiospore, 12
Beans as culture medium, 172
Beck, C., 200
Berlese, A. N., 50
Bessey, E. A., 256
Betanaphthol as antiseptic, 237
Biochemical activity, loss of, 222
Biochemical studies, cultures for, 18
Biochemistry of moulds, 247
Biourge, Ph., 99, 130, 137, 144, 175
Birkinshaw, J. H., 127, 141, 223, 2
Bisby, G. R., 66, 79, 82, 259
Biverticillata-symmetrica (sect, of *Pe*
  cillium), 134, 158
Blakeslee, A. F., 13
Boidin, A., 27
Borax as antiseptic, 226, 234
Bordeaux mixture, use of, 234
Boric acid as antiseptic, 226, 234
Botrytis, 60, 71, *80*
B. cinerea, *80*, **81**
Bottom yeasts, 48
Brefeld, O., 130
Brettanomyces, 53
Brevi-compacta (sect. of *Penicilliu*
  142, 144
Brewing, 48, 239
Brewing yeasts, 48
Brie cheese, 150, 240
Brine as preservative, 230
British Cotton Industry Research Ass
  ciation, 236
Brooks, F. T., 82, 207
Brown, W., 93
Bulbs, parasites of, 153, 154, 157
Buller, A. H. R., 52
Bunker, H. J., 258
Burgess, R., 257
Burgundy mixture, use of, 234
Burnett, Sir W., 233
Byssochlamys, *34*
B. fulva, *34*, **35**
  resistance to heat of, 34, 207, 226
B. nivea, 34

Calcium chloride, control of humidity by means of, 228
Caldariomyces fumago, 202
Caldariomycin, 202
Calmette, A., 27, 242
Calonectria, 59, 94
Camembert cheese, 129, 150, 240
Canned fruit, spoilage of, 34, 226
Canning of foodstuffs, 226
Carbon dioxide, utilization of, 239
Carbon tetrachloride for control of mites, 216, 218
Carpenteles, 130
Carrot extract agar, 213
Carrots as culture medium, 172
Carter, J. C., 210
"Cellophane", cultures on, 180
Cements for microscope slides, 195
Cephalosporium, 70, **76**, *79*, 192
Cephalothecium, 18
C. roseum, *80*
Chætocladium as parasite of moulds, 210
Chætomium, *39*
  loss of fertility in, 220
  monograph on, 39, 255
C. chartarum, 39
C. globosum, **37**, *39*
Chain, E., 245, 246, 247
Charles, J. H. V., 247
Cheese, Brie, 150, 240
  Camembert, 129, 150, 240
  Gorgonzola, 129, 240
  Roquefort, 129, 144, 240
  Stilton, 129, 240
Chinese red rice, 39
Chinese rice, 240
Chivers, A. H., 39, 255
Chlamydospore (def.), 7
Chlorine, utilization of, by moulds, 202
Chlorphenols as antiseptics, 236
Chromium compounds as antiseptics, 233
Church, M. B., 99, 100, 175, 176, 177, 255
Cider, 49
Cigarette paper for exclusion of mites, 219
Citric acid, production of, 98, 129, 241
Citrinin, antibacterial properties of, 143, 247
  production of, 143
Citromyces, 129, 134, 241
Citromycetin, production of, 141
Citrus fruits, antiseptics for, 226, 234
  control of moulds on, 226
  rot of, 142, 157
Cladosarum olivaceum, 120
Cladosporium, 71, 72, *80*, 90
C. herbarum, 59, *82*, **84**
  temperature limits of, 207
  tolerance to thallium of, 231
Clamp connections, **10**, 12
Classification, principles of, 15
Clements, F. E., 253
Clutterbuck, P. W., 149, 202, 245
Coccidiascus, 44
Coghill, R. D., 245
Coles, A. C., 190, 200
Colin-Russ, A., 235, 237
Collette, A., 27
Colour production by moulds, 203, 204, 207
Columella (def.), 20
Condensers for microscope, 190
Conidiophore (def.), 7
Conidium (def.), 7
Connstein, W., 243
Control of moulds by antiseptics, 230
  by limitation of available moisture, 227, 230
  by physico-chemical means, 227
  by sterilization, 226
  by ultra-violet radiation, 227
  on fruit, 226, 234
  on rubber, 234, 236
  on textiles, 233, 235, 236
  on tobacco, 227
Coons, G. H., 220
Copper compounds as antiseptics, 233
Coprophilous fungi, 210
Corda, A. C. I., 253
Coremigena (sect. of *Penicillium*), 161
Coremium (def.), 7
Coremium, *90*
C. glaucum, 90
C. sylvaticum, 90
Corn-meal agar, 174, 213
Cotton-wool plugs, 164
Creosote as antiseptic, 235
Crowell, I. H., 216
Cruess, W. V., 49
Culture flasks, 182
Culture media, 169
  effect of reaction of, 203
  for stock cultures, 213
  for yeasts, 54, 55, 56
Culture tubes, 164

Cultures, deterioration of, 220
  for biochemical studies, 181
  on "Cellophane," 180
  on slides, 179
  revival of old, 213
  types of, 177
Currie, J. N., 98, 241
Curtin, T. P., 202
Czapek agar, 177, 213
Czapek's solution, 176

Dade, H. A., 259
De Bary, A., 39, 256
Dematiaceæ, 64, 66, 69, 173
Debaryomyces, 44
Derx, H. G., 52, 158
Deterioration of cultures, 220
Deuteromycetes, 59
Diastase, 239
p-Dichlorbenzene for control of mites, 216
Dichloricide, 216
Dichloronaphthoquinone, 237
Diehl, W. W., 195
Dierckx, R. P., 130, 175
Dilution cultures, 184, 186
Diplostephanus, 60
Discomycetes, 33, 34
Divaricata (sect. of *Penicillium*), 142
Dodge, B. O., 40, 78
Dodge, C. W., 257
Dox, A. W., 176
Drechsler, C., 89, 249
Dry-ice, 239
Dry-rot, 208
Dual phenomenon, 221
Dunn, C. G., 258
Duske, A. E., 235

Eel-worms, destruction of, by moulds, 249
Einhorn tubes, 55
Elements essential to moulds, 169, 201
Elliott, J. A., 90, 255
Elliptica-magna (sect. of *Penicillium*), 142
Emmons, C. W., 158
Empusa muscæ, 19
Endomyces, 43
Endomycetaceæ, 34, 43
Endomycoideæ, 43
Endomycopseæ, 43
Endomycopsis, 43, *44*
E. vernalis, *44*
  production of fat by, 243
Engler, A., 254
Entomophthorales, 19
Environment, effect of, 201
Enzymes of moulds, 239
Erdin, 202
Eremascoideæ, 43
Eremascus, 43
Ergosterol, production of, 129, 244
Erysiphales, 34
Eurotium, *39*, 59, 60, 107
E. Chevalieri, 17
Exoascales, 34
Eyepieces, 189

Factory hygiene, 225
Family names, 17
Fasciculata (sect. of *Penicillium*), 1
  153
Fasciculation in *Penicillia*, recogniti
  of, 137, 153
Fat, production of, by moulds, 243
Fawcett, H. S., 226
Fawns, H. T., 244
Felix, E. L., 237
Fermentation tests on yeasts, 55
Filtration of culture media, 171
Fink, H., 243
Fitz, A., 239
Fitzpatrick, H. M., 257
Fleming, A., 143, 181, 245
Flor of sherry, 49
Florey, H. W., 245, 246, 247
Food canning, 226
Food requirements of fungi, 201
Foot-cells in *Aspergillus*, 98, **101**
  in *Penicillium*, 99
Forceps, 166
Formaldehyde, sterilization by means o
  168
Form genus (def.), 13, 60
Form species, 60
Fruit, control of moulds on, 226, 234
  moulds causing rot of, 142, 154, 157
  spoilage of canned and bottled, 34, 22
Fulmer, E. I., 44
Fumigacin, 247
Fumigatin, 247
Fumigation of laboratories, 168
  of mite-infested cultures, 216
Fungi, classification of, 3
  distinction from other organisms
  1, 2.

Fungi—*continued*
  food requirements of, 201
  general morphology of, 3
Fungi Imperfecti, 4, 12, 59
Funiculosa (sects. of *Penicillium*), 137, 142, 152
Fusarium, 7, 59, 60, 65, 70, *90*, **95**, **96**, 173
  deterioration of cultures of, 220
  perfect stages of, 59, 94
  taxonomic literature of, 255

Galle, E., 240
Gallic acid, production of, 242
Galloway, L. D., 227, 229, 257, 258
Gametangia (def.), 11
Gaŭmann, E. A., 257
Gelatine culture media, 170, 171, 173
Genus (def.), 15
Geodin, 202
Germ-tube (def.), 3
Gibberella, 60, 94
Gladiolus corms, rot of, 153
Glass, moulds on, 202
Gliocladium, 70, 71, *133*
G. catenulatum, *133*
G. deliquescens, *133*
G. fimbriatum, 248
G. roseum, **131**, *133*
Gliomastix, 70, *79*
G. convoluta, **77**, *79*
Gliotoxin, 248
Glister, G. A., 247
Gloiosporæ, 66, 69
Gluconic acid, production of, 242
Glycerol, antiseptic value of, 209
  production of, 242
Gorgonzola cheese, 129, 240
Gorodkowa's agar, 55
Griseofulvin, 202
Group species (def.), 100
Guilliermond, A., 43, 53, 257
Gwynne-Vaughan, H. C. I., 33, 257
Gymnascaceæ, 34

Haehn, H., 243
Hagem, O., 21
Hand-lens, use of, 191
Hansen, H. N., 188, 219, 221
Hanseniaspora, 44, *50*, 52
Hansenula, 44, *49*, 53
Hansford, C. G., 82, 207
Harshberger, J. W., 21, 257
Helminthosporium, 16, 72, **85**, *89*
  inducing sporulation of, 221
  pigments produced by species of, 248
H. gramineum, pigments of, 248
Henrici, A. T., 21, 43, 49, 53, 180, 257
Herrick, H. T., 242
Heterosporium, 72, *89*
H. Magnusianum, 16
Heterothallism, 13, 39, 158, 222
Hetherington, A. C., 141, 143
Hexamine as antiseptic, 231
Hildebrand, E. M., 188
Hind, H. L., 53, 200
Hoerburger, W., 243
Hohl, L. H., 49
Homothallism, 13
Hormodendron, 82
Humidity, control of, 228
Hygiene in factories, 225
Hypha (def.), 3
Hyphomycetales, 63
  classification of, 64, 69
  key to, 69
Hypomyces, 60, 94
Hypopus, 219

Identification of species, 197
Illuminants for microscopy, 190
Incubators, 168
Indicators, mould pigments as, 204
Industrial fermentations, symposium on, 258
Isaria, 71, *90*, **91**
Isolation of moulds, 184

Jam, resistance to mould attack of, 209, 230
James, R. F., 227
Jennings, M. A., 247
Jewson, S. T., 216
Johann, H., 256
Johansen, D. A., 181
Journals, mycological, 259

Kefir, 49
Kidd, M. N., 207
Kinttof, W., 243
Klœckera, 50, *52*
K. apiculata, **51**
Kluyver, A. J., 47, 52
Koch sterilizer, 167
Koji, 240
Kojic acid, production of, 127
Koumyss, 49

Lacto-phenol., 192
Lafar, F., 240, 258
Lamps for microscopy, 190
Lanata-divaricata (sect. of *Penicillium*), 138, 142, 150
Lanata-typica (sect. of *Penicillium*), 142, 149
Langeron, M., 60, 130, 200
Latin, use of, 18
Leather, antiseptics for, 235, 236, 237
Lendner, A., 21
Lentinus lepideus, phototropism in, 207
Lichens, 1, 2
Lieske, R., 67
Light, influence on growth of, 204, 221
Lindau, G., 254
Linder, D. H., 195
Liquid culture media, 170, 178, 181
Lister, A., 2
Lockwood, L. B., 243
Lodder, J., 50, 52, 256
Longevity of moulds, 212
Loss of biochemical activity, 222
Lüdecke, K., 243
van Luijk, A., 249
Luteo-virida (sect. of *Penicillium*), 158, 161

McPherson, W. H., 44
Macrophoma, 63
Macrospores in *Pœcilomyces*, 134, **135**
Macrosporium, 40, 90
Maintenance of cultures, 212
Malt extract culture media, 173
Mangin, L., 104
"Marmite", 244
Marsh, W. S., 235
Mason, E. W., 66, 69
May, O. E., 242
Measuring oculars, 189
Media for cultures, 169
  for mounting, 192
Melanconiales, 63, 93
Melanospora, 40
M. destruens, **38**, *40*
Mercurials, organic, 235
Mercury salts as antiseptics, 234
"Merfenil" as antiseptic, 235
Methyl salicylate for control of mites, 216
Metulæ (def.), 130
Micromanipulators, 188
Micrometry, 189
Microscopic equipment, 188
Microscopical methods, 191
Microscopy, books on, 199
Microsphœerlla Tulasnei, 59
Miscellanea (sect. of *Penicillium*), 162
Mites, 215, **217**
  control of, 216
  eggs of, 215, **217**
Mixed cultures, effect on growth of, 209
Moisture, available, 227, 230
  minimum amount of, required growth, 227
  requirements for moulds, 208
Monascus purpureus, **36**, *39*
Monilia, 71, *78*
M. sitophila, 39, 59, **75**, *78*
Monosporella, 44
Monoverticillata (sect. of *Penicilli* 134, 138
Moore, W. C., 138
Morphology, general, of fungi, 3
Morris, L. E., 231, 232
Morris, M., 245
Mould, enzymes, 239
Mould-ripened cheese, 240
Mounting media, microsopical, 192
Mucedinaceæ, 64
Mucor, 13, 17, 19, 20, *21*, **23**, 191, 21
  zygospore production in, **9**
M. hiemalis, 27
M. mucedo, 22, 239
M. piriformis, 22
M. plumbeus, 22
M. racemosus, 22, **23**
  production of alcohol by, 239
M. Ramannianus, 27
M. Rouxii, 27
  use of, 239
M. sexualis, **9**
M. spinescens, 22
M. spinosus, 22, **24**, **25**
Mucoraceæ, 17, 180
Mucorales, 13, 17, 19
  classification of, 19, 20
  parasites of, 210
  taxonomic literature of, 21, 256
Mushrooms, 12
Mutation in moulds, 222
Mycelia sterilia, **62**, 65
Mycelium (def.), 3
Mycetozoa, 2
Mycoderma, 52, *53*
Mycological journals and periodicals, 2

Mycomycetes, 4
Mycotoruloideæ, 50, 53
Myxomycetes, 2

Nadsonia, 44
Nadsonieæ, 44
NAUMOV, N. A., 21
Nectria, 60, 94
Needles for sowing cultures, 166
  support for, 166
Nematospora, 44
Nematosporoideæ, 44
Neurospora, *39*, 78
N. sitophila, **37**, 78
N. tetrasperma, 78
NISIKADO, Y, 89
Nitrates, utilization of, by yeasts, 54, 56
Nomenclature, rules of, 16

Objectives for microscope, 188
OBOLD, W. L., 258
Oidia (def.), 7
Oidium, 70, *72*
OLLIVER, M., 34, 207, 226
Oogonium (def.), 8
Oomycetes, 8
Oosphere (def.), 8
Oospora, 70, *72*
O. crustacea, *72*, **73**
O. lactis, *72*, **73**
  production of fat by, 244
Oospore (def.), 8
Optical equipment of microscope, 188
Ordinal names, 17
ØRSKOV, J., 67
Osmophilic yeasts, 49, 209
Oxalic acid, production of, 98, 241
OXFORD, A. E., 202, 246, 247

Pæcilomyces, 71, *134*, **135**
  macrospores in, 134, **135**
P. varioti, *134*, **135**
PAGE, A. B. P., 216, 218
Paper for wrapping fruit, 226
  for excluding mites, 219
Paranitrophenol as antiseptic, 236
Paraphyses (def.), 33
Parasites of fungi, 210
PASTEUR, L., 234, 242
Patulin, 247, 248
Pears, rot of, 154
PEASE, D., 216, 219
Penicillic acid, antibacterial action of, 248
Penicillin, antibacterial action of, 143, 246
  production of, 246
Penicillium, 16, 59, 60, 71, 129, **131**, 210
  citric acid production by, 241
  classification of, 129, 134
  conidium formation in, 130, **131**
  diagnosis of, *130*
  difficulty of taxonomy of, 129
  disease of *Aspergillus niger*, 161, **206**, 211
  distinction from *Aspergillus*, 99
  gluconic acid production by, 242
  group species in, 137, 138
  instability of species of, 137
  taxonomic literature of, 256
  temperature optima of, 208
P. asperulum, *144*
P. avellaneum, *158*
P. biforme, *150*
P. brevi-compactum, *144*, **148**
  biochemical test for, 149
  production of phenolic substances by, 149
  thermal death-point of, 207
P. brunneo-violaceum, **155**
P. camemberti, 129, *150*
  use of, 240
P. camemberti var. *Rogeri*, *150*
P. canescens, *152*
P. caseicolum, *150*
  use of, 240
P. Charlesii, 138, **139**
P. chrysogenum, *143*, **147**
  antibacterial activity of, 246
P. citrinum, *142*, **145**
  production of citrinin by, 143, 247
P. claviforme, 90, 247
P. corymbiferum, 154, *157*
P. cyclopium, *154*
  production of penicillic acid by, 247
P. Daleæ, 16, *152*
P. digitatum, **140**, *142*, 157
  control of, 226, 234
P. Duclauxi, *161*
P. expansum, 90, 137, *154*, **156**, 248, 249
  control of, 226
P. flavi-dorsum, *142*
P. flexuosum, *157*
P. frequentans, **140**, *141*
P. funiculosum, **160**, *161*
P. glabrum, *141*
P. gladioli, *153*
P. Gorgonzola, 144

P. griseo-fulvum, 202
P. italicum, *157*, **159**
  control of, 226, 234
P. Janczewskii, *152*
P. janthinellum, *150*, **151**
P. javanicum, production of fat by, 243
P. lanosum, 16, *150*
P. luteo-viride, *161*
P. luteum, **136**, *158*
  series, *162*
  deterioration of cultures of, 220
P. minio-luteum, 161
P. nigricans, **151**, *152*
P. notatum, *143*
  production of penicillin by, 143, 245
P. olivaceum, *142*
P. oxalicum, *142*
P. patulum, 248, 249
P. puberulum, *143*
P. purpurogenum, 16, *162*
P. Raistrickii, **145**, **146**
P. roqueforti, 16, 129, *144*, **147**
  use of, 241
P. rugulosum, *161*
  as parasite of *Aspergillus niger*, 161, 210
P. sanguineum, *161*
P. sclerotiorum, 202
P. spiculisporum, *158*
P. spinulosum, **136**, *141*, 247
  biochemistry of, 141
  loss of biochemical activity in, 223
P. Stilton, 144
P. stoloniferum, *149*
P. suaveolens, 16
P. terrestre, *152*
P. Thomii, *138*
P. urticœ, *157*
P. viridicatum, *154*, **155**
P. Wortmanni, *158*
Penicillus (def.), 130
Perithecium (def.), 33
  distinction from pycnidium, 40
Perry, 49
Petri dishes, 165
  use of, 178
Phenol as antiseptic, 236
Phenyl mercuric nitrate as antiseptic, 235
Phialides (def.) 8
Phoma, *63*, 220
P. pigmentivora, *153*
P. terrestris, **61**
Phototropism in fungi, 204
Phycomyces, 20, *31*
P. nitens, *31*
  phototropism in 31, 204, **205**
Phycomycetes, 4, 17, 257
Phyllosticta, 63
Pichia, 44, *49*, 53
Pigments of *Helminthosporia*, 247
  of moulds, effect of aeration on, 2
  — — of effect of light on, 2
  — — of effect on reaction medium on, 204
Pilobolus, phototropism in, 207
Piptocephalis, 11
  as parasite, 210
Pityrosporium, 52
Plants, classification of, 1
Plating-out, 184
Plectascales, 34
Plectomycetes, 34
Plenodomus fuscomaculans, 220
Pleospora, 40, 90
Plugs for culture tubes, 164
Poisons for fungi, 209
Polyzime, 98, 240
PORTER, C. L., 210
Potato agar, 173
  for stock cultures, 213
Potato plugs, 172
POVAH, A. H. W., 21
PRANTL, K. 254
PRESCOTT, S. C., 258
Progametangia (def.), 11
Pronunciation of names, 17
Proper names as names of species, 16
Proteins, production of, 53, 244
PRUESS, L. M., 245
Prune agar for stock cultures, 213
Prunes, use of, for culture media, 172
Puccinia de Baryana, 16
Pullularia, 70, *78*
P. pullulans, **74**, *78*
Purification of cultures, 186
Pycnidium (def.), 7
  distinction from perithecium, 40
Pyrenomycetes, 33, 34, **38**, 39
Pyridine for control of mites, 216
Pythium, inhibition of, 249

RABENHORST, L., 3, 254
Radiata (sect. of *Penicillium*), 143
Raistrick, H., 89, 127, 129, 141, 143
  202, 223, 247, 249
Rami (def.), 130
RAMSBOTTOM, J., 248, 257

ANDLES, W. B., 200
APER, K. B., 107, 112, 255
AULIN, J., 174, 201
aulin-Dierckx medium, 175
aulin's medium, 174
aulin-Thom medium, 175
eaction of medium, effect on growth of, 203
EILLY, J., 202
REINKING, O. A., 94, 255
Relative humidity, control of, 228
RENDLE, T., 226
Respiration of fungi, 202
Restricta (sect. of *Penicillium*), 143
Rhizopus, 4, 16, 19, 20, *28*
  use of species of, 31, 239
R. arrhizus, *31*
R. japonicus, *31*
R. nigricans, 4, **5**, **10**, 11, **26**, *28*
  influence of light on, 204
R. tonkinensis, 31
Rhodotorula, *50*, 52
Rhodotorulaceæ, 50
Roquefort cheese, 129, 144, 241
Roux bottles, 183
Rubber, antiseptics for, 234, 236
Rusts, 12

SACCARDO, P. A., 21, 60, 64, 65, 198, 254, 255
Saccharification of starch, 98, 239
Saccharomyces, *44*, **46**
S. carlsbergensis, *47*
S. cerevisiæ, **45**, *47*, 53, 55, 244
S. cerevisiæ var. *ellipsoideus*, *47*, 50
S. fragilis, *49*
S. Kefir, *49*
S. pastorianus, *48*
Saccharomycetaceæ, 43
Saccharomycodes, 44
Saccharomycoideæ, 43
Saké, 98, 240
Salicylanilide as antiseptic, 236
Salicylic acid as antiseptic, 236
  utilization of, by *Aspergilli*, 231, 236
SALTER, C. T. C., 258
Saprolegnia, 8
SARTORY, A., 107
SASS, J. E., 181
Scab, 67
Schizoblastosporion, 52
Schizosaccharomyces, 42, 43, *44*
S. octosporus, *44*
S. Pombe, *44*, **54**
Schwanniomyces, 44
Sclerotiorine, 202
Sclerotium (def.), 8
Scopulariopsis, 71, *133*
S. brevicaulis, **132**, *133*
  arsenic poisoning due to, 133
  spoilage of cheese by, 241
S. brevicaulis var. *alba*, *133*
S. brevicaulis var. *glabra*, 133
Sectoring in cultures, 222
Septum (def.), 4
SHAFIK, M., 216, 218
SHEAR, C. L., 40, 78, 130, 253
Shellac cement, use of, 56, 195
SHERBAKOFF, C. D., 256
SHIMWELL, J. L., 56
Shirlan as antiseptic, 236
Silica gel, use of, 230
SIMONART, P., 202
Single-spore isolation, 186
Slide cultures, 179
Slides, preparation of, 192
Slopes, 177
SMITH, A. L., 2
SMITH, G., 34, 99, 143, 181, 202, 247, 248, 249
SMITH, H. F., 258
Smuts, 12
SNYDER, W. C., 219, 221
Sodium silicofluoride as antiseptic, 234
Soil fungi, 248
SOPP, J. O., 130
Sordaria, **38**, *40*
Sowing liquid media, 178, 182
  petri dishes, 179
  slopes, 177
Soy sauce, 240
Species (def.), 15
Sphæropsidales, 63
Spicaria elegans, 134
Spinulosin, 247
Sporangiole (def.), 31
Sporangiophore (def.), 20
Sporangium (def.), 8
Spore (def.), 3
Sporobolomyces, 12, *52*
Sporodochium (def.), 8
Sporophore (def.), 8
Sporotrichum, 70, **76**, *78*
Stachybo'rys, 70, *82*, **206**, 210
S. atra, *82*, **85**
Stains for moulds, 193
Stansfield, J. M., 247
STANTIAL, H., 55

- Starch, saccharification of, 98, 239
- Steam sterilization of air, 168
- Stellata (sect. of *Penicillium*), 144
- Stelling-Dekker, N. M., 43, 44, 47, 256, 257
- *Stemphylium*, 72, 78, **88**, *90*
  - deterioration of cultures of, 220
- Sterigmata (def.), 8
  - methods of production of, 99, **101**
- *Sterigmatocystis*, 99
- Sterilization, intermittent, 171
  - of air, 168
  - of culture media, 167, 171
  - of glassware, 166
  - of metal tools, 166
- Stevens, F. L., 89
- Stilbaceæ, 64
- Stilton cheese, 129, 240
- Stock cultures, contamination of, 214
  - culture media for, 213
- Stroma (def.), 8
- *Stysanus*, 70, *90*
- *S. stemonitis*, *90*, **92**
- Sub-culturing, frequency of, 212
- Substage condensers, 190
- *Syncephalastrum*, 20, *32*
- *S. racemosum*, **30**, *32*
- Synthetic culture media, 174

- Takadiastase, 96, 240
- Takamine, J., 240
- Tanner, F. W., 257
- Tattersfield, F., 216
- Ter Horst, W. P., 237
- Temperature for storing cultures, 213
  - relationships of moulds, 207
- Terminology, 15
- Textiles, antiseptics for, 233, 235, 236
- Thallium salts as antiseptics, 234
- Thallophyta, 1, 2
- *Thamnidium*, 20, *31*
- *T. elegans*, **29**, *32*, 180
- Thaysen, A. C., 245, 258
- Thom, C., 99, 100, 103, 107, 112, 129, 130, 134, 137, 143, 144, 175, 176, 177, 203, 240, 255, 256
- Thymol, use of, for sterilization of air, 168
- Timber, preservation of, 233, 235
- Toadstools, 12
- Toluquinone produced by *Pen. spinulosum*, 141, 223
- Top yeasts, 48
- *Torula* Persoon, 50, 70, 71, *72*
- *T. herbarum*, *72*
- *Torula* Turpin, 50
- Torulaceæ, 50
- *Torulaspora*, 44
- Torulopsidaceæ, 50
- Torulopsidoideæ, 50, 52
- *Torulopsis*, 50, 52, *53*
- *T. utilis*, *53*
  - production of protein by, 244
- *T. utilis* var. *major*, 244
- *T. utilis* var. *thermophila*, 244
- *Trichoderma*, 70, *79*
- *T. Koningi*, 79
- *T. lignorum*, *79*
- *T. viride*, **77**, *79*
  - effect of, on other fungi, 80, 210
- *Trichothecium*, 71, *80*
- *T. roseum*, *80*, **83**
- *Trigonopsis*, 52
- Tuberculariaceæ, 64
- Tubes for cultures, 164
- Types of cultures, 177

- Ultra-violet radiation, fungicidal e of, 227
- Underkofler, L. A., 44

- Van Niel, C. B., 52
- Variation in cultures, 220, 221, 222
- Vegetable culture media, 172
- Velutina (sects. of *Penicillium*), 138, 142
- *Verticillium*, 70, *80*, 192
- *V. cinnabarinum*, **77**, *80*
- *V. glaucum*, *80*
- Viscose film, cultures on, 180, 181
- Vitamins, production of, 245
- Vuillemin, P., 65

- Wakefield, E. M., 66
- Waksman, S. A., 67, 177, 247
- Walter, F. G., 48
- Ward, G. E., 243
- Wekmer, C., 99, 222, 240, 241
- Weindling, R., 210
- Welch, F. V., 199
- Westling, R., 130
- Weston, W. A. R. Dillon, 221
- White, E. C., 246
- Wiesner, B. P., 247
- Will, H., 50
- *Willia*, 49
- Wiltshire, S. P., 90
- Wine yeasts, 47

Wollenweber, H. W., 93, 94, 255, 256
Wood, preservation of, 230, 233, 235
Wort culture media, 173
  use of, for stock cultures, 213

Xerosporæ, 66, 70

Yabuta, T., 124
Yeager, C. C., 194
Yeast extract for culture media, 173
Yeasts, 41
  ascospores of, 42, **46**, 54
  asporogenous, 42, 50
  bakers', 48
  bottom, 48
  brewing, 48
  ergosterol from, 244
  fermentation tests on, 55
  general morphology of, 41
  methods of identification of, 53
  methods of propagation of, 41, **45**
  microscopical examination of, 56
  osmophilic, 49, 209
  protein in, 244
  taxonomic literature of, 256
  top, 48
  true, 42
Yuill, E., 120, 194
Yuill, J. L., 120

Zaleski, K., 130
Zinc chloride as antiseptic, 233
Zoospores (def.), 8
Zycha, H., 21, 256
Zygomycetes, 4, 8, 17, 19
Zygorhynchus, 8, 20, *27*
Z. heterogamus, *28*
Z. Moelleri, **25**, *28*
Zygosaccharomyces, 47, 49, **51**
Zygospore (def.), 11
  formation of, **9**
Zymase, 239

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